













# RECENT ADVANCES IN MEDICINE

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## CHAPTER 1

### COMPUTERS IN MEDICINE

THE past decade has seen a continuously expanding application of digital computers in almost every field of human activity; in defence, in business, in physics and engineering, and in other sciences ranging from astronomy and meteorology to chemistry, microbiology and medicine.

#### PRINCIPLES OF COMPUTER OPERATION

Computers can be divided into two classes: digital and analogue. The digital computer operates directly on numbers as we do in performing arithmetic tasks. By coding letters into a numerical form, the digital computer can also handle alphabetical information. The versatility of the digital machine allows it to carry out virtually any mathematical task or to perform any logical set of instructions, while the "memory" of the modern machine can store vast quantities of information with almost immediate retrieval.

The analogue computer does not work with numbers. It operates on continually varying quantities. A slide rule is the simplest example, in which the quantities to be computed are represented by lengths. In the electronic analogue computer, they are represented by voltages arranged to alter in exactly the same way as the quantity being computed, and for this reason analogue computers cannot handle alphanumeric (i.e. alphabetical or numerical) data directly.

Whatever the application, the computer is concerned with the processing of data in some form or another. The "data" or raw materials of the computer consist of information of any variety whereby communication is established between human beings.

In medicine, information from a patient may be alphabetical as in notes, or numerical from the laboratory. Data may be generated in a graphical or analogue form such as in an electrocardiogram, electroencephalogram, temperature chart or blood pressure tracing. Again, information may be in pictorial form from the photography, pathology or X-ray departments. Any of these varieties of data may be fed, after modification, into a digital computer.

"Processing" of data refers to the operations which the computer performs. Thus it may sort, count, arrange, compare, calculate or predict optimum conditions in a problem. It possesses the ability to alter its own course of actions. Calculation includes ordinary arithmetical processes, elaborate statistical routines, or complex mathematical operations. Because of its inherent versatility, the computer can do many different and diverse jobs and the same machine which could print out the pay-roll for hundreds of



employees in a time measured in minutes, might also solve in a comparable time, a series of non-linear mathematical equations not previously attempted by hand because the time required might be measured in years.

Industrially, an important corollary of processing is already being achieved, whereby the results, or output of the computer is being applied to automatic control. A machine tool or even an oil refinery may be run automatically under the guidance of a computer which also initiates the necessary courses of action to continue the process. Such ideas are being extended to the automation of biochemistry laboratories, and are likely to be extended to other areas of hospital activity.

### Development of Computers

It is interesting to reflect that the principles of our modern computers were laid down with uncanny accuracy over a century ago by the Cambridge mathematician Charles Babbage. The essential features of versatility and the ability to "decide" a course of action were inherent in his proposals for an "analytical engine".

Babbage's idea of conveying information by means of punched cards or tape still forms one of the principal means of communication with a computer.

Hollerith, a statistician in the U.S. Census Bureau in 1887, again adopted the idea for a calculating machine. The census was taken every ten years, and he realized that the population expansion was such that with clerical methods alone, insufficient time would be available to allow one census to be analysed before the next was due. He coded data on punched cards and by passing them through a machine which sensed the holes, specific counting operations could be effected at greatly increased speeds.

<u>PERSON</u>	<u>LIGHTS</u>	<u>BINARY</u>	<u>DECIMAL</u>
NONE	● ● ●	000	0
HP 1	● ● ○	001	1
HP 2	● ○ ●	010	2
HP 3	● ○ ○	011	3
HS 1	○ ● ●	100	4
HS 2	○ ● ○	101	5
R 1	○ ○ ●	110	6
R 2	○ ○ ○	111	7

FIG. 1.1. Binary Arithmetic as illustrated by a 3-light hospital call system.

A further feature of the punched cards is the close relationship it bears to the form of arithmetic with which digital computers operate. A simple example serves to illustrate. A "call" system, common in hospitals, consists of a set of lights in each room. In Fig. 1.1, three lights serve to call one of seven people and provide a "no call" condition. Although it might seem simpler to use seven lights switched in sequence—coding shows this to be

unnecessary. If we designate a light "off" by 0 and a light "on" by 1, then clearly the sequence of 0's and 1's can be used for counting. This is an example of binary arithmetic in which only two symbols are required. Despite advances in electronics, the only counting operation with sufficient reliability for computing is that in which a circuit is in one of two opposite conditions, of which one can be designated "0" and the other "1". Clearly the punched card or tape accords naturally with the system of binary arithmetic and the data contained in it is sensed and passed on as a series of electrical impulses. The binary code is also used for representing the letters of the alphabet.

The method of handling graphical information is shown by reference to Fig. 1.2, which represents a waveform of arterial pressure. The level of pressure is sampled successively at equal time intervals and the shape is represented by the series of ordinates  $P_0$ ,  $P_1$ ,  $P_2$ , etc. These ordinates may be stored as numbers in a digital system, together with the corresponding time instants of

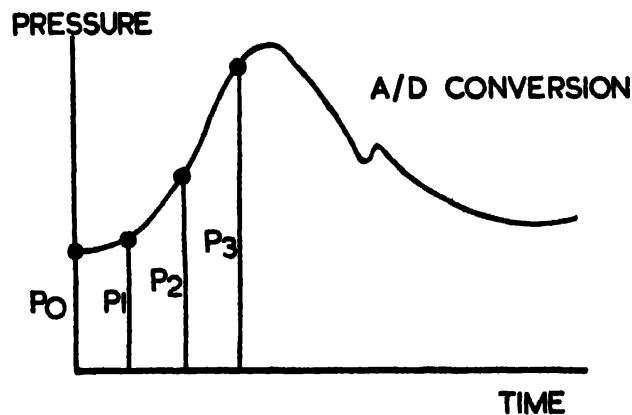


FIG. 1.2. Analogue to digital conversion by periodic sampling of an arterial pressure waveform.

measurement. In order that information is not lost, the sampling frequency is arranged to be of the order of twice that of the highest harmonic in the original waveform. In the present example, the sampling frequency would be about 50 cycles/second, although for ECG tracings, up to 1,000 samples/second have been employed. This method of processing graphical records is called analogue-to-digital (A/D) conversion.

X-ray photographs have been stored in digital computers by an optical scanning process. Imagine the picture to be divided up into a very large number of small squares and that the "blackness" in each square is measured. The numbers representing blackness can be stored together with the information regarding the position of each square. In practice this result is achieved by scanning through the picture with a narrow beam of light which falls on a photoelectric cell, the electrical output of which is related to the blackness of each element of the picture. The picture may be recovered as an image on a television screen.

The processing of cards may be carried out by a variety of machines. Depending on their degree of complexity they use the punched hole to add, subtract, divide or multiply. They may produce lists, classify, select, print out alphanumerically or in code, or punch another card. Such operations have been provided for many years in business machines. Even so, data

processing on cards alone is limited in speed and in range of operations and complexity of calculations available. The digital computer is the natural evolution of the punched card machine.

### THE DIGITAL COMPUTER

In principle, the digital computer resembles the ordinary desk calculator, but as a result of a "program" of instructions, it can carry out automatically the activities of the operator.

The essential parts of an automatic computer are shown in Fig. 1.3, and consist of:

1. A control unit to enable the computer to perform the desired operations in the correct sequence as specified by the program.
2. An arithmetic unit in which signals (electrical pulses) can be manipulated, usually according to the rules of binary arithmetic.
3. A store or memory unit for holding data and instructions, usually coded as binary digits.
4. Input unit devices, such as keyboard machines, punched cards or tape; whereby data and instructions are supplied to the machine.
5. Output unit devices, such as automatic typewriters, high speed printers which operate a line at a time, graph plotters, oscilloscopes or punched cards for displaying the results of a calculation or analysis.

Fig. 1.3 allows the sequence of events to be visualized. The program, or instructions for a given set of operations, is transferred to punched cards or tape and "read" by the input unit into the computer store. The data on which

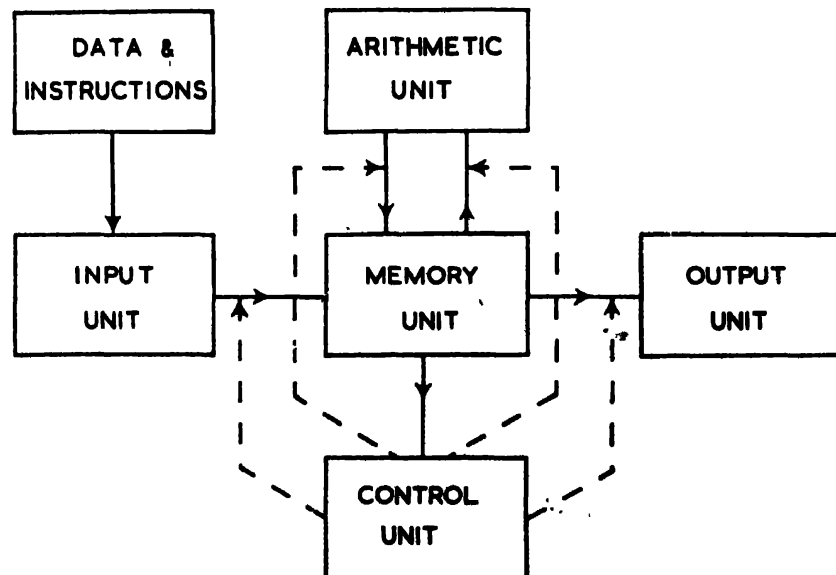


FIG. 1.3. Generalized plan and scheme of operation of a digital computer.

the operations are to be performed are similarly fed in. The first instruction is sent to the control unit for temporary storage and when completed is replaced by the next. The items of data to be computed are passed in turn to the arithmetic unit where the result of each operation is generated and then

returned to the storage unit. When all the instructions are carried out, the results are fed to the output unit.

### Computer Stores

Present-day computers usually employ several forms of magnetic store. Three types are in common use. Within the computer itself the so-called "core store" consists of many thousands of tiny magnetic rings or ferrite cores, each of which can be magnetized to saturation in one or other of two directions in order to store a binary digit as "0" or "1". They are "written" and "read" by electric currents passing along wires threaded through them. The time taken to transfer a number from store to arithmetic unit or vice versa is related to the "access time" of the store and in a typical core store may be 1-2 microseconds. For this reason, the core store is often referred to as the rapid access store.

In contrast, computers also utilize "backing stores" which may consist of magnetic tape systems similar in principle to the domestic tape recorder, or may take the form of a set of magnetic discs, like gramophone records. The magnetic medium is used in a similar manner to punched tape. However, the "hole" is now a confined region of magnetization and a very high density of equivalent "holes" may be achieved. The access time for a magnetic tape might be many seconds depending on the position occupied by a particular piece of information. Thus the items can only be reached sequentially, necessitating tape winding. For the disc, where access is random, one item of information might be picked out from half a million in about one-tenth of a second. These backing stores are used as filing systems, to store computer programs or the past records of a business or hospital, and they may be used to supplement the core store during extended computations.

The increasing speeds of digital computers bears an important relationship to present-day ideas on their modes of operation. It has been pointed out (Hollingdale, 1966) that while the input and output speeds have increased by 10 in the past decade, computer speeds have increased by 100. Various methods are being adopted to avoid wasting the time of the main computer by making it wait for the slow input and output devices. One method consists of using ancillary computing equipment additional to the main computer, simply for transcribing information from one physical medium to another.

A more recent concept is to arrange that the computer carries out several tasks at the same time such as computing on one program whilst "reading in" or "writing out" on another. This method of working is known as "time sharing". The best known example is Project MAC (Multiple Access Computer) at Massachusetts Institute of Technology. At present, up to 24 users can use the system simultaneously. Project MAC was demonstrated to an audience in Oxford in 1964, by direct connection with a telephone line across the Atlantic (MRC Publication, 1965).

The application of this important concept to a hospital is evident in which each area of activity, such as medical records, biochemistry, patient monitoring, X-ray department, pharmacy, etc., might have simultaneous communication with a central computer (Baruch, 1965).

### Programming the Computer

*Reference has already been made to the binary code of arithmetic as the language which is understood by the computer. Instructions are also coded as a series of 0's and 1's and stored as digits so that they can be manipulated in the arithmetic unit just like numbers. Earlier computers were programmed using this binary system although it is difficult to handle and inconvenient. During the late 1950s, several "high level" languages were introduced such as FORTRAN, ALGOL, and COBOL, which have revolutionized programming. Such languages are easier to learn than the cumbersome binary codes of earlier days. Nevertheless, the computer itself still requires these codes, and programs have been devised which translate the high level language into machine language. These translation programs, called "compilers" are stored in the computer before the instructions are read in.*

At the present time compatibility between different makes of computer is incomplete and a program written for one machine will not necessarily run on another. Languages such as Fortran not only simplify programming but they are contributing to the possibility of moving towards a small number of universal languages applicable to all digital computers.

Modern computers must thus be supplied with four kinds of programs:

1. Organizational programs to deal with such matters as access to the peripheral units, batch processing, time sharing, record keeping and the diagnosis of programming errors.
2. Compilers for translating from high level languages (FORTRAN, ALGOL, etc.) into the machine languages of the particular computer.
3. A library of commonly required "standard" programs which might deal with such operations as Fourier analysis, the manipulation of matrices and standard statistical calculations.
4. Programs written for specific jobs.

Programs of the first three types must be permanently kept in the store of the computer and form part of the complete installation, and are collectively known as "software". At least as much effort is now being directed to software development as to the design of computer equipment (the hardware). Particularly in medicine such effort is essential.

The necessary preparation before a problem can be run off a computer has been emphasized (Michie, 1964). Thus at least five categories of person are involved.

1. The medical or research worker who must give a clear, full and explicit description of the problem and indicate how he wishes it to be tackled. It is at this stage that considerable ingenuity has been exercised in the more successful examples of medical computing.
2. The systems analyst who must give a complete specification of the proposed method of analysis, probably including an outline in flow-diagram form.
3. The programmer, who writes and tests the program specified by the applied mathematician.
4. The keyboard operator who will transfer the program and the data to paper tape or punched cards.

5. The computer operator, responsible for running the machines and maintaining libraries of tapes and discs.

*Although it is possible for one person to function in all five roles, practical planning should make provision for the separate skills, and the decision to install a computer in a hospital or medical school entails support from an adequate research and development group.*

### MEDICAL RECORDS

Hospital records are usually kept in files numbered sequentially. Thus a hospital of some 500 beds may easily have accumulated half a million sets of case notes in the records department. While it is relatively easy to abstract any particular record from the store, cross correlation between case notes is time consuming, to the extent that it may be impossible without some arrangement for data processing. For example, if one wished to find out the duration of stay in hospital of all patients who had undergone operation for acute appendicitis or the types of complication to which this procedure was subject, during the previous ten years, a large number of records would have to be sorted by hand. However, the potentialities for clinical investigation are obvious.

Although digital computers have often been acclaimed as the solution to problems of keeping medical records, earlier efforts to reproduce and update, in digital magnetic form, the complete medical case history proved unsuccessful. The means whereby medical record keeping may be automated has been discussed (Smith, 1964). Further problems have also been outlined (Schental, Sweeney & Nettleton, 1960; Schental, 1961, 1963; Korein, Woodbury & Goodgold, 1963). It has been pointed out (Benjamin, 1965) that there seems to be general agreement that recording the complete narrative medical history of the patient in digital magnetic tape form, as a routine clinical service, is neither feasible nor economic.

Attention has therefore been more directed towards partial retrieval and such systems are widely in use. Several different approaches can be distinguished in relation to the patient's progress through hospital. Thus information may be:

- (a) Collected as it is generated and entered more or less automatically.
- (b) Entered after short delays of about a day.
- (c) Abstracted from the post-discharge summary.
- (d) Collected for application to specific areas of medical interest.

These approaches may be considered in more detail:

#### **Immediate Data Collection—Hospital Automation**

A preliminary approach to the collection, processing, storage and retrieval of hospital information has been described (Baruch, 1965). A teletypewriter is installed in each hospital area related to the patient, e.g. ward, operating theatre, X-ray department, pathological laboratory, diet kitchen, pharmacy, etc. Each item of information is entered as it arises and is fed into a central computer. This not only stores the data but a two-way communication system is established with the computer.

### **Systems with Delay**

Records generated in the ward, laboratory or other department are returned daily to a central office and there fed into a computer system, usually by coding on to punched cards or paper tape. The patient's record is updated by the computer which can then assist in diagnosis or treatment by calculations or by comparison with accumulated information in the computer memory.

A data processing system of this type has been investigated (Bennett & Holland, 1965). Information on each patient was coded on special forms which could be "read into" the computer by a photoelectric device. A medical and a surgical firm co-operated in the experiment. For each patient, identification, together with diagnostic and operative details were recorded. Additional specialized forms were included as indicated. For example, the surgical firm, mainly urological, produced a form for cystoscopy findings and for other special investigations. The forms, procedures and methods employed also permitted the preparation and handling of case summaries.

### **Post Discharge Systems**

The preparation and storage of a summary of the case history when a patient is discharged was adopted in certain British hospitals in the early 1930s. Some ten years later several undergraduate teaching hospitals were entering these records on punched card systems. The main objects (Benjamin, 1965) were (1) to provide an index of diagnosis and operations and to facilitate the retrieval of specific case histories and (2) to indicate patterns of morbidity in the hospital from data based on diagnoses, and to provide certain indices, e.g. duration of stay, whereby the management of clinical services could be evaluated. Hospitals now return this information to the Ministry of Health on a national basis.

At the London Hospital, much of this work within the medical records department has been advanced by replacing the previous punched card system by digital computer processing. Information identifying the patient, mode of admission, diagnosis, operations, etc., allow a post discharge summary to be prepared by the computer. Various administrative statistics can be prepared regarding length of stay in relation to each consultant, bed occupancy, number of closed and extra beds, information on emergency admission of patients, outpatient admissions of new and old patients, and progress is being made in applying these data to improve hospital services in relation to the known needs.

More elaborate post-discharge systems are in operation in certain centres in the United States, particularly at Ann Arbor (Slee, 1961), where this type of information is centrally processed on behalf of some 200 hospitals.

### **Special Applications of Records**

Many efforts have been directed to the automation of records in specific situations such as postmortem findings (Smith & Melton, 1963; Bahn, Schmit & Young, 1965). The College of American Pathologists has produced a special code for the digital coding of pathological findings (College of American Pathologists, 1964). Biochemical laboratory findings have also been recorded (Jurgens & Rosevere, 1964; Smith & Melton, 1963).

A computer program for compiling anaesthetic records has been outlined (Hagelsten & Bennike). Items are recorded relating to type of operation, duration, pre-operative complicating factors such as asthma, diabetes, anaemia, allergy, abnormal ECG or chest X-ray. Anaesthetic factors relate to premedication, intubation, relaxants, type of anaesthetic and technique, and include many factors concerned with complications. The advantage of analysing large numbers of cases for research and improving methods is pointed out.

Several studies have been reported in obstetrics and up to 90 items of information were collected on each of 300,000 births from 150 hospitals in 1962 (Kane, 1963, personal communication). The information was processed and a complete analysis of the data was returned monthly to each hospital.

### **Record Linkage**

The aim of record linkage is to bring together separately recorded facts about an individual or family. The potentialities of collecting such information over wide geographical areas has been discussed (Newcombe, 1965; Spicer, 1965). The Oxford Record Linkage Study was commenced in 1963, with the object of building up a central file of information about health in which the basic unit was the individual. The progress of this scheme has been reported (Acheson, 1965) and is discussed in detail in Chapter 13.

## **THE COMPUTER IN THE LABORATORY**

### **Automation in Biochemistry**

The last decade has seen an enormous increase in the demands made on the routine hospital biochemical laboratory. The annual rate of increase in the number of analyses reported from a number of teaching hospitals is of the order of 15 per cent, i.e. doubling every five years (Whitby, 1964; Moss, 1965). This expansion not only relates to the increase in number of any one type of analysis but also to the growing diversity of investigations which are of clinical significance.

The increased volume of work presents numerous problems of laboratory organization and considerable advances are already being made by the application of automatic equipment both for chemical analysis and for data processing.

The way in which such advances may be implemented has been outlined (Wootton, 1965). He points out that a simple flow diagram of work going through a laboratory may be represented as in Fig. 1.4. Specimens and request forms generated in the ward reach the laboratory where they are separated. The sample is investigated at the point marked "analysis" on the chart. The result, calculated in acceptable units, is eventually married to the patient data in order to produce the final report, usually after an editing stage is interposed. This stage is important, for as the basis of the general knowledge of the editor, unacceptable results and inconsistencies in the data may be rejected. The simple flow sheet may clearly be extended and an additional stage, often regarded as essential, is to include reference to the store of previous results as part of the editing process. For example, rapid and excessive change in a given biochemical value would require explanation.



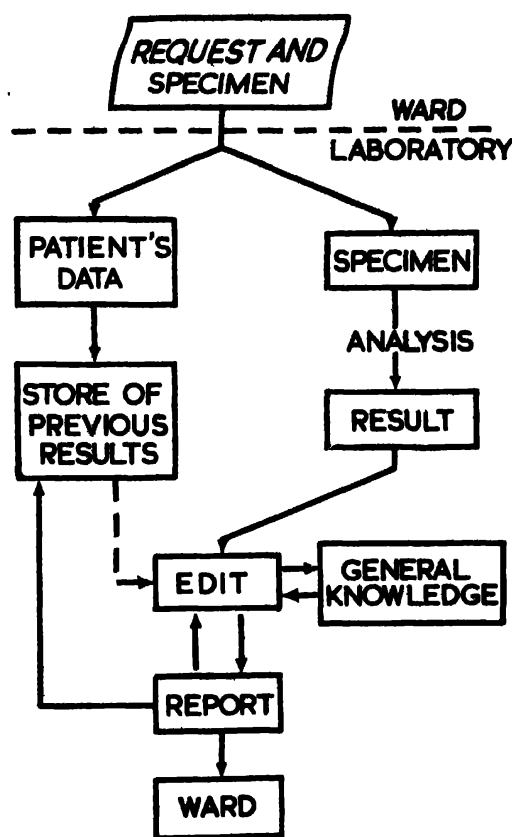


FIG. 1.4. Laboratory flow chart for clinical biochemistry.

The expansion of biochemical requirements within a flow scheme such as in Fig. 1.4 inevitably means a considerable increased load of clerical work and this in part is borne by the skilled scientific staff. It has been shown (Whitehead, 1964) how data processing equipment can assist. The objectives of the system were firstly to reduce the clerical staff and secondly to produce computer orientated digital data as a by-product of the office routine. The information on the request form is punched into the first 48 columns of a punched card, and includes the patient's name, registration number, ward, age and sex, together with the required test and specimen number. The cards vary in colour according to the group of tests required.

Simultaneously, with the operation of card punching, a day sheet, listing all the specimens arriving on that day, is automatically typed. The punched cards are then sorted according to the tests required and work sheets are typed out which show the list of specimens required for each analysis. On completion of the test, the laboratory worker enters the result on the work sheet which when completed is returned so that the result may be punched into the card. The card is then used to prepare the report. The information on the card may be stored in a computer.

Not only is automation beginning to play an important part in the organization of the laboratory, but automatic methods for chemical analysis are becoming common in biochemical laboratories.

It has been shown (Flynn, 1965) that automatic analysis still leaves the formidable task of processing, correlating, checking and distributing all the

information so obtained and the advantages of the digital computer for this purpose have been shown. Flynn has applied analogue-to-digital conversion equipment to the recorders of the AutoAnalyzer, at present by far the most widely used automatic equipment. Automation applied to chemical analysis not only results in considerable time saving, but eliminates the inevitable human errors involved in reading the peaks manually.

The developments which have been outlined are summarized in Fig. 1.5, which also serves to emphasize other advantages of the digital computer used in association with the laboratory. The request may be in the form of a pre-scored punched card in which a pencil may be used to push out the pre-scored section next to the test required. In this way a punched hole is produced without special equipment. Such cards may be read directly into

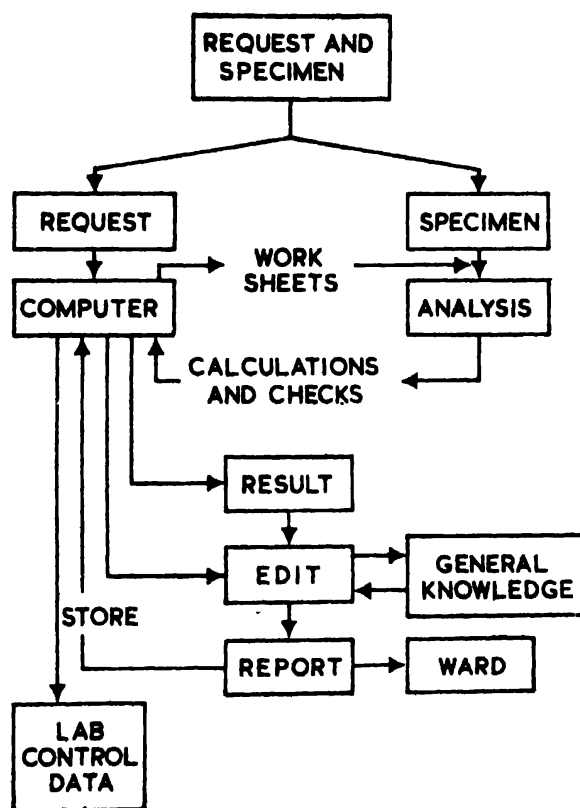


FIG. 1.5. Laboratory flow chart for clinical biochemistry using a computer.

the computer which can generate work sheets which serve to order the specimens for automatic analysis. From the AutoAnalyzer, the raw data may be fed to the computer for further processing to produce a result. The development of colorimeters and flame photometers with digital outputs further assists entries into the computer. By the use of the computer, the editing process can be expanded (Wootton, 1965). Thus while the analysis is being carried out, the request is used to interrogate the store regarding previous results on the patient and their relevance to the new results being produced. The computer can inform the editor of a selected proportion of the stored results. For example, if a blood urea is being performed, previous blood urea results would be relevant, especially those of recent origin, as would also be the previous values of serum potassium and bicarbonate. On

the other hand, previous bilirubin or glucose results would not usually be needed.

Another advantage of the computer relates to the tabulation of test results for scientific purposes. By conventional means, it is difficult or impossible to analyse the records from large numbers of patients, to establish future needs from current trends or to assess the efficiency of staff deployment. Whitehead (1964) has discussed such analyses in relation to laboratory control. An example is given in Fig. 1.6 of variations in mean blood urea values, and illustrates the use of cumulative sum charts (cusum). In such a chart an arbitrary "mean" value is chosen and the difference between the actual means and the arbitrary "mean" is plotted cumulatively. For example,

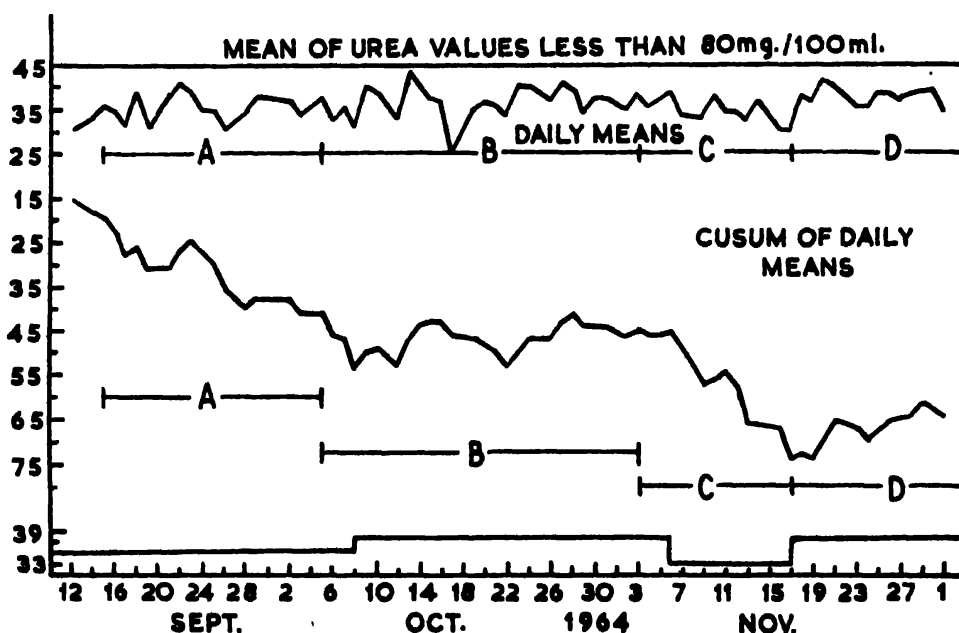


FIG. 1.6. Cusum analysis of daily means of blood urea.

if the arbitrary "mean" was 30 mg/100 ml., and on successive days the actual means were 33, the cusum graph would be 3, 6, 9, etc. on succeeding days, i.e. the graph would be a straight line of uniform slope. Figure 1.6 shows that four distinct slopes occurred over a three-month period. These slopes corresponded closely to the periods when four different laboratory workers were operating the AutoAnalyzers. Clearly, methods of this kind, which require the use of computers to handle the considerable quantities of data, will ultimately allow much fuller and more precise use to be made of biochemical analysis.

### Molecular Structures

One of the outstanding uses of digital computers in the past few years has been to elucidate the structure of molecules of biochemical importance (Ledley, 1965). X-ray crystallographic studies have provided detailed configurations of penicillin, vitamin B<sub>12</sub>, myoglobin and haemoglobin, while the determination of DNA has provided an indispensable link to the understanding of protein synthesis and the genetic code. Working out the structure of these very large and complex molecules involves an enormous number of

computations which would be impossible without the concurrent development of the digital computer.

## CLINICAL APPLICATIONS

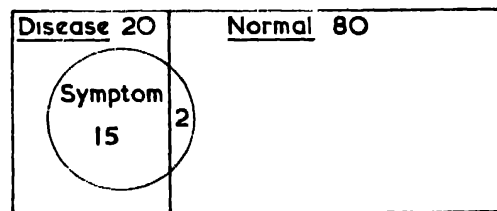
### Diagnosis

Four main methods are being applied to computer assisted diagnosis:

- (1) Conditional Probability.
- (2) Likelihood Ratio.
- (3) Numerical Taxonomy.
- (4) Multivariate Analysis.

### Conditional Probability

We normally associate a series of symptoms and physical signs with a given disease. The diagnostic process requires the reverse, as for example with the use of French's "Differential Diagnosis", namely considering a list of the possible diseases associated with each symptom. We require to



Probabilities	Symbol	Number	%
Disease in population	P(D)	20 in 100	20
Normals in population	P(N)	80 in 100	80
Symptom in Disease	P(S/D)	15 in 20	75
Symptom in Normals	P(S/N)	2 in 80	2.5

### DIAGNOSIS P(D/S)

Probability of patient having disease because he has the symptom is 15 in 17 or 88%.

But 15 is 75% of 20% of pop<sup>ln</sup>. i.e.  $P(S/D) \cdot P(D)$

2 is 2.5% of 80% - -  $P(S/N) \cdot P(N)$

$$\text{i.e. } \frac{15}{17} = P(D/S) = \frac{P(S/D) \cdot P(D)}{P(S/D) \cdot P(D) + P(S/N) \cdot P(N)}$$

FIG. 1.7. Bayes' formula.

know two factors. Firstly the frequency with which a given symptom is associated with each disease in the group under consideration and secondly the frequency with which each disease occurs in the population. Given these frequencies, or probabilities, the diagnostic probability, that is the most likely disease to give rise to the observed signs and symptoms, may be calculated on the computer by a method using Bayes' formula. This is a simple arithmetic device for manipulating probabilities and it is illustrated by Fig. 1.7.

Taking a simple situation with one disease, suppose that in a population of one hundred, 20 people have the disease and 80 do not. Then we can immediately write the first two simple probabilities which occur in the table. Namely, that of a person having the disease is 20 per cent in the given community while obviously the probability of a person not having the disease is 80 per cent. Now consider one symptom related to the disease; suppose that the symptom is illustrated by the circle. As shown, let 15 diseased people have the symptom and two people in the normal group have it also. Then two further probabilities are available in the table. Thus the probability that the symptom occurs, given that the disease occurs, is 15 in 20 or 75 per cent. This is the incidence of the symptom in the disease. Furthermore, the probability that given the symptom, the patient is normal, is 2 in 80 or 2½ per cent. The latter two are conditional probabilities, meaning that P is the "probability that" the first symbol occurs given that the second symbol occurs. We now require the diagnostic (conditional) probability that the person is diseased, provided that he exhibits the symptom. Obviously from Fig. 7, this is 15 in 17, or 88 per cent. Can this be calculated from the probabilities given in the table? Obviously it can for:

15 is 75 per cent of 20 per cent of the population

$$= P(S/D) \times P(D)$$

2 is 2½ per cent of 80 per cent of the population

$$= P(S/N) \times P(N)$$

$$P(D/S) = \frac{15}{17} = \frac{P(S/D) \times P(D)}{P(S/D) \times P(D) + P(S/N) \times P(N)}$$

In this expression the important quantity is the numerator for the denominator represents the total number of people with the symptom in the community and this can be regarded as a normalizing factor.

Although this example refers only to one disease and one symptom, it may be generalized by taking the sum of all such terms as the products in the numerator and the denominator. This method has been used (Warner *et al.*, 1961) for the diagnosis of congenital heart disease, using some 60 symptoms and physical signs and 33 diseases. This study shows that there are certain problems in the application of the method.

(1) The symptoms must be independent in a given disease. Cyanosis and clubbing are so often found together as to suggest that they are causally related. Only cyanosis was included in Warner's study.

(2) Diseases should be mutually exclusive. Congenital heart disease is usually classified anatomically. If pulmonary stenosis and atrial septal defect occur together the symptoms of the combination would not be predictable from the separate conditions, because of the increased likelihood of shunting of blood from the right to the left side of the heart. Thus although cyanosis may occur in some 1 per cent of pulmonary stenoses and perhaps 2½ per cent of atrial septal defect, with the combination present, it can occur in 20 per cent. This is not predictable from the separate conditions so that the combination must be made equivalent to a separate disease.

(3) The occurrence of a disease in a community depends on place, season, epidemics and so on, and this occurrence is required for the model.

(4) The Bayes' Model will only diagnose those conditions for which the model is set up.

By careful choice of symptoms, it has been found (Toronto, Veasey & Warner, 1963) that, in a limited analysis of 36 cases, the computer diagnosis agreed with that made by physiological studies and observations at operation, at least as often as the diagnosis, given as most probable, by three experienced cardiologists. The program has been further evaluated, modified and expanded. It has recently been reported (Warner, 1966) that of all patients seen in his clinic, some 10 per cent are able to avoid cardiac catheterization as a result of the predictions of the computer.

A similar model has been used in the diagnosis of Cushing's syndrome (Nugent, 1964). By means of Baysean analysis, data from 772 psychiatric patients has been used to obtain a preliminary diagnostic classification (Bernbaum & Maxwell, 1960). Probability theory techniques have also been applied to the differential diagnosis of obstructive jaundice (Bykhovsky, Vishnevsky & Kharnas, 1961; Bykhovsky & Vishnevsky, 1962). A model has been devised (Lipkin *et al.*, 1961) for the differential diagnosis of 20 hæmatological diseases based on a standard textbook classification. Baysean analyses have also been used in the diagnosis of disorders of thyroid function (Overall & Williams, 1963; Fitzgerald & Williams, 1964). They determined the most prevalent clinical characteristics in 879 patients with thyroid disease. They then classified them as hypothyroid, euthyroid or hyperthyroid, using their response to treatment over at least six months as the criterion for classification, i.e. a therapeutic diagnosis. This information allowed a Baysean probability matrix to be set up and a further 268 patients were used to estimate the probability with which each patient fitted into one of the three groups. In 96 per cent, the therapeutic diagnosis was correctly predicted.

Other examples of conditional probability models have been given in the diagnosis of bone tumours (Lodwick, 1963) and in the investigation of epigastric pain (Renaldo, Schlinok & Rupe, 1963).

### **Likelihood Ratio Method**

This method has been applied to a public health project at the Kaiser Permanente Clinic in California (Collen *et al.*, 1964). The object is to use multiple tests to rule out the presence of a large number of diseases in patients visiting the clinic. It is known as multiphasic screening. The tests are carried out before the patient is seen by the physician and they include a wide variety of investigations which take some 2-2½ hours. The tests include an ECG and phonocardiogram, a table tilt cardiovascular test, and height, weight and body measurements which are automated. In addition, chest X-ray and mammographies are carried out. Various tests of vision include visual acuity, ocular tension and a retinal photograph. Further tests for hearing, a general health questionnaire, and various investigations of blood chemistry, hæmatology, serology and a urine analysis are done. The test information is so arranged in steps so that only yes/no answers are necessary. No assumptions are made about independence of symptoms.

To see how the method works, take a simple example. Suppose that answers are obtained to three dichotomous questions. There are eight possible answers or symptom combinations. In general if  $n$  symptoms

(questions) are selected, then 2<sup>n</sup> combinations result. We want to know which combination is most closely related with a given disease. Figure 1.8 illustrates this simple example, and we see that 74 people out of 100 gave the answer "yes/no/no" to the three questions. If they all have a particular disease we can establish the probability which this combination is associated with the disease,  $P_D^s$  as 74 per cent. However, this complex will also arise in some people who are not diseased. Suppose that this incidence

LIKELIHOOD RATIO  
QUESTIONNAIRE  
Diseased Population

A \ Q	1	2	3	No eg
1	Y	Y	Y	2
2	Y	Y	N	0
3	Y	N	Y	4
4	Y	N	N	74
5	N	Y	Y	1
6	N	Y	N	3
7	N	N	Y	5
8	N	N	N	11

Y = Yes      N = No

FIG. 1.8. Likelihood ratio table applied to investigation of disease in a population.

is also found and is  $P_N^s$ , then the ratio of these probabilities is the likelihood ratio.

$$\theta = \frac{P_D^s}{P_N^s}.$$

The possible symptom complexes may be listed in ascending order of likelihood ratio and a decision is made from accumulated statistics as to the level

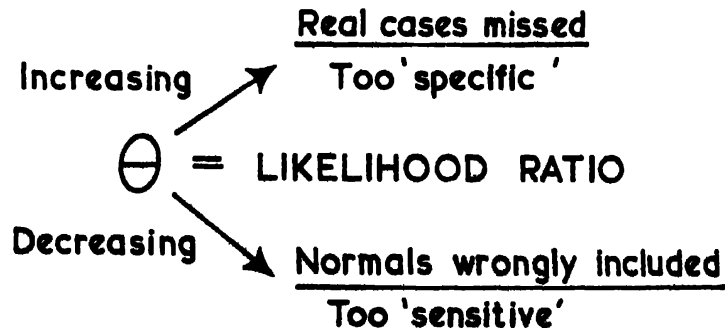


FIG. 1.9. Effect of varying likelihood ratio on incidence of false positives or false negatives.

at which the likelihood ratio should be set to affect a diagnosis. This raises further problems, illustrated in Fig. 1.9. Suppose the likelihood ratio is set to a high value. What this means, in effect, is that we have an almost idealized

“text book” situation in which only those cases which closely conform to a particular symptom complex are rated as having the disease. It is well known that a vague symptomatology, with many factors apparently missing, does not rule out a diagnosis. In other words, the test is too specific and will miss actual cases. This is called an error of the first kind or a false negative.

Again if the likelihood ratio is set too low then although we accept a more diffuse symptomatology and miss relatively few real cases, we include persons who do not have the disease. This is called an error of the second kind or false positive, and in these circumstances, the test is too sensitive. The likelihood ratio can be adjusted to a level which gives the best compromise between specificity and sensitivity. Obviously in the case of a lethal disease such as tuberculosis, where we would want to miss very few cases, the sensitivity could be set at 99 per cent or more, when only one in a 100 real cases would be missed, but this would be at the expense of including more normal people.

In considering why a computer is needed for this type of analysis, it can be pointed out that for 20 “yes/no” questions, more than a million combinations arise, whereas if we increase the number of questions to 32, we can get some 4,000 million answers.

### Numerical Taxonomy

This method and its possible application to the study of liver disease has been described (Baron & Fraser, 1965). A preliminary report has been given on 50 such patients, each represented by some 300 characters which include history, symptoms, signs, biochemistry, histology—in fact as complete clinical information as possible (Fraser & Baron, 1966).

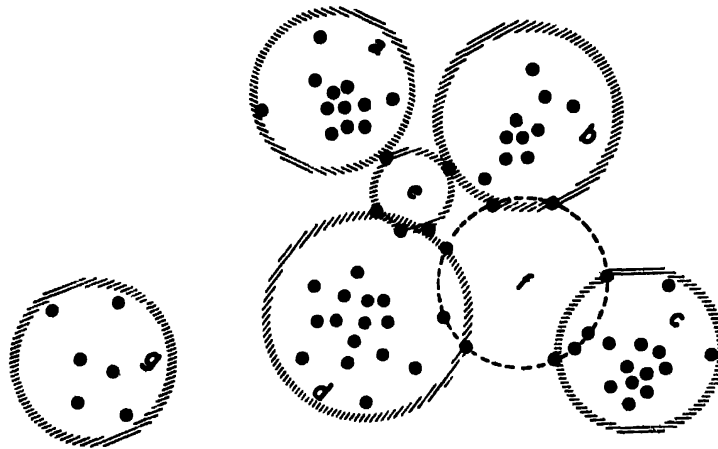


FIG. 1.10. Two dimensional representation of the distribution of patients in a multidimensional space, as derived theoretically by a cluster analysis technique of numerical taxonomy. (From BARON, D. N. & FRASER, P. M., 1965. *Lancet*, 2, 1066.)

Thus if there is reason to doubt that an ideal division of a set of related disorders into disease entities has been reached, an objective analysis can be made by applying the techniques of numerical taxonomy. These were devised originally for bacteriological classification and plant taxonomy. The great difficulty with this type of procedure is the problem of weighting important characters against unimportant characters. No assumptions are made as to the existence of any particular disease entities, and the characters



for each patient are compared with those for every other patient. The relation between any two patients is expressed numerically as a similarity coefficient, which is calculated as the ratio between the number of characters in common between two patients and the number of characters possessed by either or both. A matrix of coefficients is thereby obtained and a cluster analysis then groups together patients who are most similar. The clusters are distributed in a multidimensional space, and those which are sufficiently isolated may be regarded as disease entities.

In Fig. 1.10 groups a, b, c and d, represent known diseases. The diagnostic groupings found by computer analysis may or may not coincide with those normally used. The method may reveal an unsuspected relation between some patients who had hitherto been placed in different diagnostic groups (e)—primary aldosteronism, for example, was unrecognized as a separate entity until available information was carefully reassessed. On the other hand, a diagnosis used at present may be applied to patients who, on careful analysis are found to resemble each other less than they individually resemble members of other groups (f)—an example of this is the disorder described as infectious lymphocytosis occurring as part of many other infections.

Once the disease entities have been newly defined, weight can be given to the characters of greatest diagnostic significance. By using the probability methods already described, a new patient can then be compared with every disease entity. However, if a new patient is compared with the complete original matrix of patients and assigned to the appropriate cluster, and the information in his case is added to the matrix, the disease register will be continually brought up to date. This technique could reveal a change in disease patterns. A group of patients might emerge who resembled each other more than they resembled any existing cluster; they would then have to be considered as a new disease entity (g). In this way, the thalidomide disaster for instance may have become apparent earlier than it was, had a central computer facility been available for recording neonatal abnormalities together with the drugs taken during pregnancy.

These techniques have been used to classify the acute leukæmias on the basis of cytology and cyto-chemistry (Hayhoe, Quaglino & Doll, 1964). Blood and bone marrow smears from 140 consecutive patients with acute leukæmia were examined and it was found that the computer analysis defined four groups, characterized by eleven principal features ("characters"). Fifty patients remained unclassified, but further statistical analysis enabled almost all of them to be included in the four groups.

An attempt has been made to clarify the diagnosis of pyelonephritis (Zinsser & Bonner, 1962). Their sample consisted of 350 patients cross-tabulated against 800 different variables ("characters"). Their analysis revealed three well-defined clusters in 115 patients and a residual set of 235 unclustered patients. They were able to define the salient features for each cluster and to demonstrate that the disease commonly diagnosed as pyelonephritis had many facets.

### **Multivariate Analysis**

The statistical procedures of multivariate analysis have been applied not only to ~~generate~~ patients into disease groups but also to determine which

characters, or set of characters, will contribute most effectively to the separation. Such studies might usefully follow taxonomic analysis.

An example of the use of multivariate analysis has been provided (Steinburg *et al.*, 1961; Caceres & Rikli, 1961). The object of their study was to see how well an individual could be fitted into his disease group using information resulting from measurements on the patient (Fig. 1.11). An electrocardiogram (lead V<sub>5</sub>), a ballistocardiogram (acceleration), a phonocardiogram (mitral area) and a radial arterial pulse were recorded simultaneously from 15 normal subjects, 15 subjects with left ventricular hypertrophy (LVH) due to hypertension, and 15 with LVH due to aortic incompetence (AI).

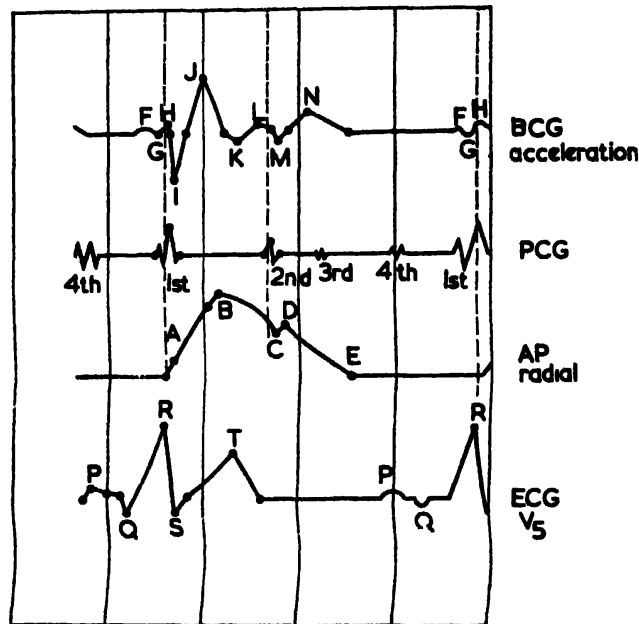


FIG. 1.11. Simultaneous measurements of ballistocardiogram (BCG), phonocardiogram (PCG), arterial pulse (AP), and electrocardiogram (ECG), as factors used for multivariate analysis.

Various parameters were measured, comprising the amplitude and time intervals of all the features of each recording. These, together with the systolic blood pressure, the pulse pressure, the age and weight of the subject, made up 45 variables. The computer carried out a multivariate analysis as a result of which it was possible to indicate for each patient a likelihood ratio for the presence of either hypertension or aortic incompetence. In addition, those factors which were most powerful in effecting the separation became evident. The results indicated that one of the hypertensive patients was classed with the non-hypertensive group, and three patients out of 15 with AI were classed in the hypertensive group.

This study is of interest because a considerable degree of segregation of cases was effected without specific medical knowledge and on the basis of relationships between variables which are not generally recognized clinically.

Other examples of this method include an attempt to find the best method of diagnosing suspected cases of primary carcinoma of the lung and bronchus (Hollingsworth, 1959). It was found that 14 factors obtained from symptoms,

signs, radiological and cytological examinations could in themselves provide 85 per cent efficiency in diagnosis in 200 cases. Data from 520 patients with systemic lupus erthematosus have been analysed (Dubois & Tuffanelli, 1964), the clinical manifestations, prognosis and causes of death summarized and the frequency of combinations of various diagnostic criteria ascertained.

### ECG Interpretation

Various approaches are being made to the interpretation of the ECG. The tracing is subject to A/D conversion and stored as a complex of amplitudes and times in the computer. Initially, the measurement of the trace is similar to the method of the human reader. A reference point is taken from which the measurements of amplitude and time intervals can begin. An obvious choice would be a point on the isoelectric base line, but this is variable.

In Fig. 1.12, the rates of change of amplitude, i.e. the "first derivative" of the ECG is formed. The thick line shows a formalized ECG as a series of straight lines. Note that the start of the P wave or Q wave etc. is marked by a sudden change of the derivative which can be used to detect beginnings and

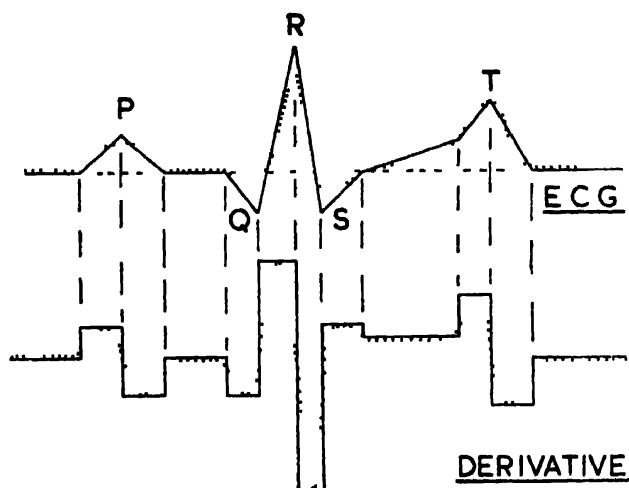


FIG. 1.12. ECG analysis by formation of the first derivative.

ends of P, QRS, and T waves. Again the dotted curve of a more real ECG shows a most negative point in the derivative which is the steepest slope of the ECG (usually the R wave) and this is taken as the starting reference. From this point, every amplitude, e.g. height of P, QRS, and T, shape of ST segment, duration of P, PR, QRS, and T can be measured in the computer and printed out. Allowance is made for base-line drift.

For diagnosis, criteria are first set up concerning how the various parameters measured are distributed between different forms of heart disease, and the computer calculates the probability with which an unknown trace fits these. The details differ—for example, measured information has been used to compute eight instantaneous cardiac vectors for each of P, QRS, and T waves by Pipberger. He has prepared information on the distribution of vectors from thousands of records of known conditions. He uses multivariate analysis to correlate the unknown with the known groups,

and works out probability density factors which indicate the extent to which the unknown group fits the known, yielding diagnosis (Pipberger, 1965).

In investigations of this type, factors such as age, height, sex and ECG, related drugs are included for diagnosis. These systems can be on-line with a local hospital group and the ECG technician can communicate directly with the computer.

Many comparisons have been made between conventional and machine assessment. For example, a series of 134 old myocardial infarcts which had occurred a year or more prior to reassessment was reported (Pipberger, 1965). Conventional 12 lead assessment—by eye—showed evidence of the infarct in 70 per cent cases. The computer diagnosed infarction in all cases.

### Patient Monitoring

The extension of ECG analysis to monitoring, for example, of patients with coronary artery disease is obvious. An analysis every  $3\frac{1}{2}$  seconds over a 14-hour period has recently been performed (Caceres, 1966) and the detailed variations of the ECG plotted more or less continuously.

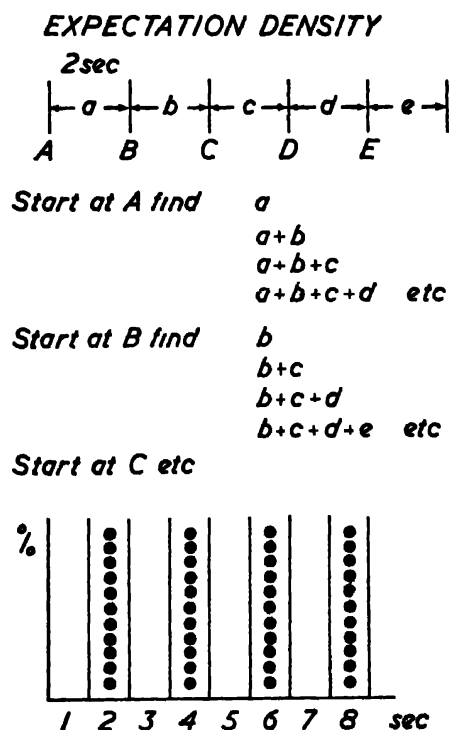


FIG. 1.13. Principles of Expectation Density analysis.

Interest in recent years has increased in the management of patients having acute myocardial infarction and it is usually agreed that some form of continuous oscillographic monitoring is desirable. For effective measures of treatment it is important to assess the presence of arrhythmias and extra systoles, and obviously to detect immediately the onset of ventricular fibrillation, if resuscitation is to be effective.

It has been pointed out (Sayers, 1966, personal communication) that

methods of signal analysis are available for assessing the extent and nature of irregularities which might occur in a series of cardiac cycles. One such method involves the use of the expectation density function. This measures the fraction of times in a record that any event, such as an R wave, occurs at a chosen time after another R wave. The way in which the method is applied is illustrated by the simple numerical example of Fig. 1.13.

Suppose that a series of time intervals is taken such as  $a$ ,  $b$ ,  $c$ ,  $d$  etc. Starting at  $A$ , calculate the time intervals  $a$ ,  $a+b$ ,  $a+b+c$ , and so on. Each time interval is scored in the appropriate "box" shown; each "box" in this example represents successive one second intervals. It is immediately obvious that if the original intervals  $a$ ,  $b$ ,  $c$  etc., are all equal to two seconds, then peaks build up in the histogram plot in the even number time interval boxes. On the other hand, if all the intervals were different there would be no preference and the histogram would be "flat". However, suppose that more intervals of two seconds were present than any other interval. It is found that peaks occur at two-second intervals in the histogram but they decrease in height as one proceeds along the time axis of the histogram.

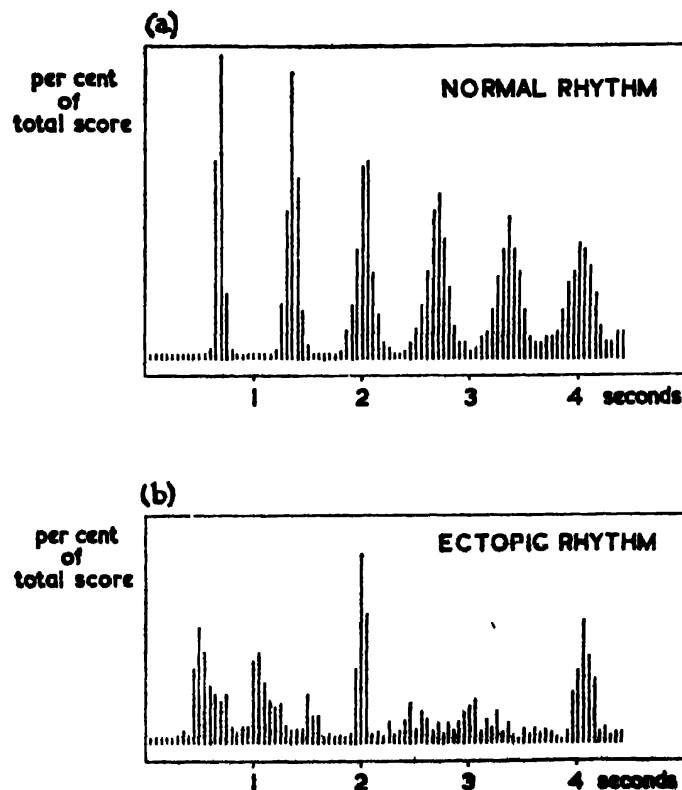


FIG. 1.14. (a) Expectation Density function for normal heart rhythm.  
(b) Expectation Density function for abnormal heart rhythm (N.B. the peaks at half second intervals are interleaved with those at 2 second intervals).

This situation is shown in Fig. 1.14(a) which is the computer plot of the expectation density function for the beat to beat intervals of a normal heart. The essentially regular character of the cardiac cycle is apparent.

However, if two cyclic events combine in an interleaved way, both sets of intervals are apparent. If in addition to the sinus pacemaker, another

Independent focus appeared, the cardiac events might become very irregular, even though both pacemakers performed fairly regularly. In Fig. 1.14(b), Sayers has shown the result for the abnormal heart in a boy undergoing cardiac surgery for a congenital abnormality. The expectation density plot shows a periodicity of about 0.5 seconds, interleaved with a two-second interval.

The examples shown in Fig. 1.14, utilize only a single parameter of the ECG, but as Caceres has shown, many ECG parameters can be derived. All these could be used separately or combined to form indices which might prove useful for trend detection. The results could be cross correlated with other simultaneous physiological recordings.

It has recently been shown (Warner, 1966) how cardiac catheterization laboratories have been linked "on-line" with a digital computer. Intra-cardiac pressures, oxygen saturations and outputs are recorded "on-line" and computations on these are immediately available in the investigation room. For example, mathematical models may be devised to provide information regarding cardiac contractility or the elastic properties of the aorta, and the equation for these can be fitted to the measured data to provide further insight into the extent of a given condition. Such studies may well be closely related to prognosis, particularly in relation to cardiac surgery.

In the more general situation, a computer system linked with an acute care ward, whereby ten variables are continuously available and updated values can be typed out on demand, has been described (Weil, 1966).

The value of these developments has not yet been fully assessed but the trends toward medically desirable information, accurately and more or less continually generated, cannot be underestimated.

### Analysis of the Electroencephalogram

As early as 1875, Caton showed that when the terminals of a galvanometer are connected to two points on the human scalp, the instrument undergoes ceaseless oscillations (Caton, 1875). The earliest analyses of the EEG indicated that it was characterized by a continuous spectrum of frequencies from under 1 c/s to 50 c/s. More recently, it has been recognized that much of the purposeful activity occurs in an apparently random background.

Analysis of the EEG is concerned with detecting periodicities or patterns contained in the signal and trying to relate these to physiological activities or to diseased states, so that the information may be presented in recognizable form.

Various techniques are employed to search for recognizable patterns in the EEG. One application has been reported (Brazier, 1965) in the treatment of temporal lobe epilepsy. The object is to detect the pattern of "spikes" or seizure discharges in the EEG activity in the region of the hippocampus or amygdala.

Needle electrodes are inserted into the temporal lobe and allowed to remain for two weeks or more. The computer is asked to detect spikes in the deep sites and to indicate whether one focus of discharge acts as a "trigger" to set off other foci. The therapeutic step is merely to carry out coagulation through the "trigger" electrode. While opinions differ as regards the appli-

cation of surgery in these cases, and obviously further assessment is necessary, it may well be that computer assisted treatment could improve on the radical operation of temporal lobectomy.

### Evoked Responses

A further field in which computer analysis is being applied is to elucidate the response evoked in the EEG following the application of periodic stimuli to the central nervous system. Averaging computers are being employed to enhance "signal to noise" ratio. Following the original method (Dawson, 1954), many workers have applied evoked response techniques in clinical situations; for the detection of abnormalities in the visual system (Brazier & Barlow, 1956; Vaughan & Katzman, 1963, 1964) and of cysts interfering with the auditory system (Brazier, 1962). The impairment of hearing has been studied by means of an analogue computer (Lowell *et al.*, 1961). The use of implanted electrodes has allowed the effect of anaesthetics and muscle relaxants on visual stimuli in man to be investigated (Domino, Corsen & Sweet, 1963).

### SIMULATION STUDIES

Considerable attention has been directed in recent years to the simulation of biological systems by computers. A number of earlier simulations of parts of the cardiovascular system were directed towards comparisons between alternating current theory and the properties of hydrodynamic flow systems. While agreement between the observed system and the model was often surprisingly close, the simpler models frequently represented necessary but not sufficient conditions for the operational features of the biological system. The alternative is to determine the mathematical model to which the actual physiological observations may best be fitted.

An interesting example of the use of this technique is provided by work in respiratory physiology. Fry has constructed a model of the relationships between flow, volume and intrathoracic pressure changes during normal respiration, and the method consists essentially of calculating the best fit of surfaces to the observed respiratory data. Most clinicians are familiar with the calculation of a regression line relating two quantities. Sometimes the regression line is not straight, but is some kind of curve. Elaborate mathematical procedures are available for fitting non-linear regressions. When the number of variables is increased a multidimensional plane or surface may be computed, but clearly the process of multiple regression analysis is considerably more complex than is the simple problem with two variables. The computer is especially useful for this purpose.

These ideas are being used to produce statistical models not only of lung mechanics but also of cardiac parameters, with such variables as instantaneous left ventricular flow, intraventricular pressure and wall tension. It has been pointed out (Fry, 1960) that many of the properties of the cardio-pulmonary system are beyond the limits of measurement by present instrumentation, but they may be inferred mathematically from more simply measured parameters. The mathematical model which will fit the physiological system is usually extremely complex and requires the use of a computer to derive numerical solutions.

Another simulation study, in biochemistry, has been carried out (Maloney *et al.*, 1963). They have simulated the biochemical system of blood by means of a model in which the distribution of the chemical constituents of the plasma, the erythrocytes and the alveolar gases have been represented. The principle is to suppose that the constituents are in chemical equilibrium. The thermodynamic condition for equilibrium of such a system is that its free energy shall be a minimum. A series of equations may be written, of which the following is a simple example:



The free energy of formation of each substance is known. If such equations, which are not independent, are solved simultaneously for the minimum free energy condition, the solution yields the concentration of each component.

The equations formulated in this study represented the major chemical reactions of plasma and erythrocytes, including those involving proteins, haemoglobin and the alveolar gases and electrolytes. Restrictions on the solution were provided by the thermodynamic conditions of the law of conservation of mass and by the Donnan membrane equilibrium conditions for providing electrical neutrality in separate compartments. Allowance was made for the sodium pump mechanism of the cell. The computer prints out in milli-equivalents per litre the major constituents of the plasma, cells, and alveolar gases. The problem is solved for steady-state equilibria without regard to chemical changes occurring in time. When the computer solutions are compared with the laboratory findings the biological system must be sampled before interaction with the surroundings, such as renal excretion of electrolytes, has occurred. With this reservation, the computer produced results in good agreement with the laboratory findings in a variety of problems concerned with addition alkalosis and acidosis, overhydration, adjustment to abnormal respiratory gases and so on. Because the equations are temperature dependent, they can be used to predict results in hypothermia. Oxygen dissociation curves can be printed out for any temperature, pH, haemocrit or alveolar gas tension.

Discrepancies between the computer and the laboratory findings have mostly been attributed to laboratory errors. For example, trouble was traced on separate occasions to a partially occluded jet in a flame photometer and to bottles of specimens wrongly labelled.

Further examples of computer simulation will be found in the texts referred to in the bibliography.

### THE ANALOGUE COMPUTER

It has been pointed out above that the electronic analogue computer does not work with numbers, but that it operates on continually varying quantities which are represented within it by voltages, arranged to alter in exactly the same way as the quantity being computed. In this sense the voltage is analogous to the computed quantity. Analogue computers perform the usual mathematical operations of addition, subtraction, multiplication, division differentiation and integration, directly and continuously on the waveforms presented to them, as a result of the properties of electrical circuits and without recourse to numbers.



When operating on any particular problem, it is usual to set up the whole problem at once on the analogue computer. Circuits performing multiplication work together with those for integration and so on, so that the answer is virtually instantaneous. In this sense the operation of the computer is such that all the separate parts of a calculation occur in parallel, in contrast to the serial operation of the digital machine. For this reason, analogue computers can often solve problems many times faster than their digital counterparts.

Setting up the problem is relatively simple and programming consists of connecting the various circuit elements together by inserting plugs into sockets, as on a telephone switchboard. The accuracy of computation, of about 0.01 per cent, is limited by the operation of the amplifiers and by the accuracy of components used in the calculating networks. A possible disadvantage of analogue machines is that problems may become too large for the computer. Here a reasonable compromise is necessary although quite complex physiological problems may be solved by computers with some 50 operational amplifiers.

### Comparison of Analogue and Digital Computers

In view of the essentially different modes of operation of the two types of computer, it is of interest to make some comparison between them.

The digital computer, which is characterized by sequential high speed operation, has great flexibility, precision and memory. It is, however, relatively difficult to program and therefore to change a program. It has a relatively high cost. However, it is capable of solving virtually any mathematical problem or carrying out a data processing operation which might involve the many thousands of items of a medical record.

The range of problems which can be solved by the analogue computer is more limited, but its programming is usually much easier and can immediately be carried out by the research worker. The conceptual simplicity of the mathematical sequences and the ease of changing programmes rapidly gives the operator insight into a particular problem and results in the analogue approach having a special appeal to workers in applied physiology. The combination of arithmetic and integration allows easy solution of ordinary simultaneous differential equations, and the parallel mode of operation often permits much more rapid solution than for digital computers. Although the memory facilities of analogue machines are small, track and hold devices are provided which give limited memory, and when linked with comparators, values of parameters may be stored at an intermediate stage and then used subsequently. By connecting comparator outputs to relays or solid state switches, calculations may be terminated and restarted, permitting repetitive execution of solutions which may be viewed on an oscilloscope. Modification of circuit parameters allows a theoretical prediction to be matched with an experimental result.

The ease of operation of the analogue computer gives the investigator an immediate "feel" for the physical system which the computer models, and its very high speed in many calculations, makes it particularly suitable for "on-line" computation in many experimental situations.

### **Functions of the Analogue Computer**

In its applications to medical problems, the analogue computer usually combines several of its essential functions. Before considering specific examples, the principal functions may be listed as follows:

- (a) Mathematical computation and analysis.
- (b) Data processing.
- (c) Simulation of part of a biological system.
- (d) As a versatile laboratory instrument.

### **Mathematical Computation**

The analogue computer is particularly efficient in solving simultaneous differential equations in which time is the independent variable. Such equations arise in such applications as compartmental analysis, the distribution and elimination of drugs from the body, and in cardiovascular and respiratory problems. Rates may be converted to amounts simply by connecting the electrical analogue of "rate" to an operational amplifier connected as an integrator.

Analogue computers are less convenient for solving problems involving distributed parameters, or for partial differential equations which involve two independent variables, although in certain instances these can be treated by approximation methods. Curve fitting is possible for the computer can synthesize sums of algebraic or exponential terms, and the process can be carried out under direct vision on an oscilloscope while the coefficients are manipulated to provide the best fit with experimental data.

The ease with which the computer can function either as a low, high or band pass filter makes the calculation of moving averages and variances possible.

### **Data Processing**

The analogue computer, with its poor memory facilities, is not suitable for large quantities of statistical data, as for example, in the management of hospital records.

It is, however, well suited to the performance of mathematical operations on measured variables, and can often be arranged to be "on-line" with experimental procedures. Cardiac performance has been studied by continuously measuring variables such as aortic flow and pressure and left ventricular pressure. The time integral of aortic flow allows stroke volume and cardiac output to be derived. The product of stroke volume and left ventricular pressures provides a continuous measure of cardiac power, while the integration of this parameter, stroke by stroke, yields the stroke work. Again the average arterial pressures divided by the cardiac output yields vascular resistance. Because of the operational speed of the computer, each of these quantities can be written out virtually simultaneously with the original data.

### **Simulation**

One of the principal uses of analogue computers in biology has been to set up dynamic mathematical models of a system or sub-system of the body.

The model may include differential equations and empirical relationships known to apply, and these are programmed on the computer. The same input conditions are then applied to the model as to the living system. The recorded outputs are compared and the structure of the model and its coefficients are adjusted until the output matches the recorded results from the living organism.

### The Computer as a Laboratory Instrument

To supplement existing laboratory tools, the analogue computer may be used for a variety of purposes, ranging from a low frequency oscillator, differential amplifier, logarithmic amplifier, slow sweep generator, wave shaper or filter. The ease with which conventional electronic devices may be simulated often saves considerable time and effort in the experimental situation.

## MEDICAL AND PHYSIOLOGICAL APPLICATIONS

### Cardiovascular Physiology

The various functions outlined have been combined on the control mechanisms operating in circulatory dynamics (Warner, 1962). For example, Warner has shown how the circulation (Fig. 1.15), can be represented as a

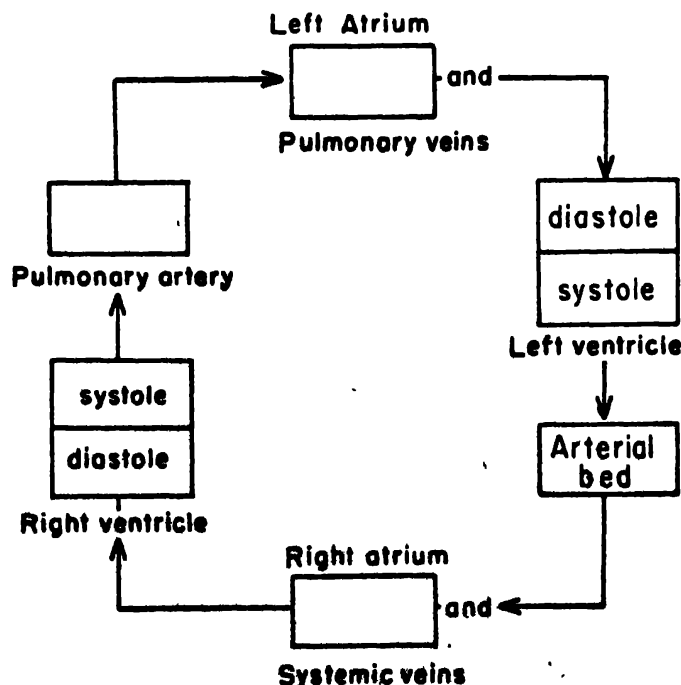


FIG. 1.15. Representation of the circulation suitable for analogue computer simulation.

closed loop with two symmetrical halves. Each half consists of a distensible reservoir (large veins and atrium), a pump which has two states, diastole and systole, and a source of resistance which succeeds the large arteries and which is located at the level of the small arteries and arterioles. Each element is affected by that element immediately preceding and succeeding it, and also by elements more remotely in the circulation. For example,

information about pressure in the large arteries is sent to the central nervous system which in turn modifies flow and peripheral resistance.

Eight equations have been proposed to represent each half of the circulation, namely the venous and arterial contributions, as regards their physical or hydrodynamic properties. The simultaneous solution of the 16 equations on an analogue computer allows certain tests of circulatory behaviour to be made. For example, the model predicts a rapid return to equilibrium with no overshoot, except at high flows, following such a disturbance as a Valsalva manoeuvre in which blood is displaced from the pulmonary circuit into the systemic circuit. This is in agreement with the response observed in the biological preparation. The model has more recently been extended (Warner, 1964) to include the control mechanisms of the central nervous system and the refined model has been applied to investigate the factors which regulate cardiac output during exercise.

In another study, Stacey & Giles (1959) have examined the properties of arteries. They have used an analogue computer to determine the mechanical properties of the arterial bed. A mathematical model for the artery has been set up on the computer into which is fed a voltage proportional to a central arterial pressure. The output is proportional to the computed peripheral pulse and the computer parameters are varied until matching is achieved. Values for arterial elasticity were determined for normal subjects and in patients with hypertension, aortic stenosis and coarctation of the aorta.

In a further study on the pulsative blood flow in arteries, Gabe has shown how a special purpose analogue computer may be used to solve the differential equation which relates the pressure gradient along an artery to the blood flow (Gabe, 1964, 1965).

### Cardiac Output Measurement

There have been several accounts of the use of analogue computers for the analysis of dye dilution curves (Skinner & Gehmlich, 1959; Moody *et al.*, 1963; Wessel *et al.*, 1964; Sekelj, Tate & Nathanson, 1966). Several special purpose computers are now commercially available for this purpose.

The principle is shown in Fig. 1.16. Imagine that the exponential downslope

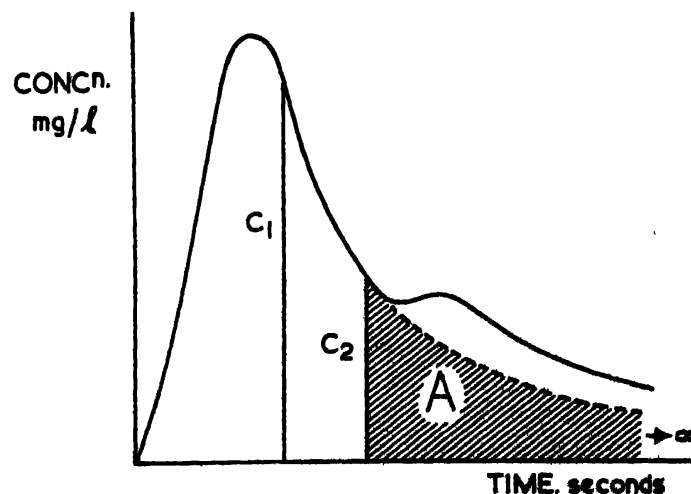


FIG. 1.16. Principle for computing cardiac output by analysis of dye dilution curve.

of the dye dilution curve is projected to infinity and the recirculation peak is ignored. The cardiac output (litres per second) is then given by the dye dose (mg) divided by the total area (mg. sec. litre<sup>-1</sup>) under the projected curve from zero to infinity. It is a geometric property of an exponential that the area  $A$  of the "tail" is directly related to the area between any two ordinates  $C_1$  and  $C_2$ . For example, if  $C_2$  is two-thirds of  $C_1$  then the tail area  $A$  is twice that between  $C_1$  and  $C_2$ . In this case, the computer, by the mathematical process of integration, calculates the area from zero to  $C_1$  and to this value adds three times the area between  $C_1$  and  $C_2$  to yield the total area from zero to infinity. By finding the reciprocal of this value and multiplying by the dye dose, the cardiac output is available at the instant when  $C_2$  is reached.

### Respiratory Physiology

A number of studies have been carried out using analogue computers in the simulation of respiratory control mechanisms. One of the first attempts (Grodins *et al.*, 1954) used the level of tissue carbon dioxide as the quantity which was being regulated and the response of this regulator to inhalation of carbon dioxide was investigated. Defares extended this model to include the role of cerebral blood flow when carbon dioxide was inhaled (Defares, Derksen & Duyff, 1960).

A model to account for respiratory sinus arrhythmia has been proposed (Clynes, 1962). The nature of the basic dynamic heart rate reflex was elucidated and it was shown that this reflex was not symmetrical for inspiration and expiration. An analogue computer was employed to test the model which was subjected to the respiratory forcing pattern. Computed and observed heart rates resulting from a variety of breathing patterns were found to be in good agreement.

A model has been devised to account for periodic breathing following hyperventilation (Horgan & Lange, 1962). A practical application of this study is to Cheyne-Stokes respiration where parameters in the model such as lung volume, blood pH, and circulation time delay can be easily varied to study the causes of periodic breathing. A similar study has been carried out in which the two compartment model of Grodins *et al.* (1954) has been extended by adding factors for circulation time and ventilatory dead space (Milhorn & Guyton, 1965).

It has been shown (McWilliam & Adams, 1963) how simple analogue computing elements may be used in plotting pressure-volume loops during the respiratory cycle, and in deducing a factor related to the collapsibility of the airways. These methods are being applied in studies in chronic bronchitis and emphysema. Studies of respiratory distress in the newborn have been carried out (Saul *et al.*, 1966) by measuring mechanical parameters of the pulmonary system. By analysing pressure-volume loops, they computed lung compliance, airway and tissue resistances together with the average rate of working during respiration to overcome the flow resistance in the system.

The use of an analogue computer for the determination of respiratory or cardiac work, particularly in patients recovering from cardiac surgery has been described (Osborn, Badia & Gerbode, 1963). An anaesthetic mask, modified to form a simple pneumotachograph, provided respiratory flow

rates while a rapid  $\text{CO}_2$  analyser measured expired  $\text{CO}_2$ . Intrapleural pressure was measured from the chest drainage tube, and the computer calculated tidal volume, tidal power and work, and work devoted to elastic and non-elastic resistance. From these results, tidal  $\text{CO}_2$  excretion and total  $\text{CO}_2$  excretion were derived.

An electrical analogue of the lung has been described (Campbell & Brown, 1963).

An interesting "on-line" application of analogue techniques in the human subject has been reported by Bellville & Seed (1959). They have developed an automatic means of obtaining carbon dioxide response curves, and shown how these vary with the respiratory depressant effects of narcotic analgesics. Murphy (1966) has extended the work of Bellville & Seed by the use of a hybrid computer in which analogue and digital techniques are used in association, to investigate the relationship between tidal volume and dead space, the effect on various ventilatory parameters of changes in posture and to the study of the relationship of the carbon dioxide response curve to respiratory control.

### Compartmental Systems

Another example of the application of analogue computers to medical problems is to the mathematical models which describe the kinetics of materials exchanging between body compartments. These systems have been described in a general way (Robertson, 1961; Randall & Metzger, 1963). They have been used to study drug metabolism (Taylor & Wigand, 1962),

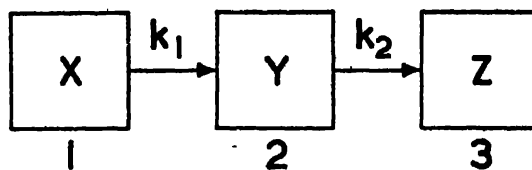


FIG. 1.17. A three compartment system: X, concentration at site of introduction; Y, in blood; Z, excreted.

the uptake of iodine by the thyroid gland (Clynes & Cranswick, 1959) and enzyme systems (Chance *et al.*, 1952; Chance, 1960; Garfinkel, 1963; Garfinkel & Hess, 1964). In a compartmented system, a biological complex is imagined as consisting, mathematically, of a number of interconnected spaces, within each of which it is assumed that perfect mixing of constituents occurs. The compartments are not necessarily contained in anatomical spaces but may be physiological, biochemical, or cellular entities.

Consider as an example, the ingestion of a drug which is introduced into the gut, passed into the blood compartment, and finally excreted. This system is represented in Fig. 1.17, in which at any time X is the amount of material at the site of introduction, Y the amount in the blood, and Z the amount excreted. At any time, assume that the rate at which the drug is taken up by the blood is proportional to the amount present in the gut and the constant of proportionality is called the "turnover" rate constant. A similar assumption will apply to the excretion of the drug. Suppose that "turnover rate constants" are  $k_1$  for the absorption constant and  $k_2$  for the excretion

constant and that we require the way in which the amount of drug in the blood varies with time.

The rate of absorption is proportional to the amount present at the absorption site at any time, i.e.

$$\frac{dX}{dt} = k_1 X$$

and  $\frac{dX}{dt}$  is shorthand for "the rate of change of X with time". Further the rate of excretion is proportional to the amount present in the blood at any time, i.e.

$$\frac{dZ}{dt} = k_2 Y$$

The rate of build-up in the blood must be the difference between the rate of absorption and that of excretion, i.e.

$$\frac{dX}{dt} = +k_1 X - k_2 Y.$$

Again, the analogue computer can be readily programmed to solve these equations simultaneously and the operator need have no knowledge of their mathematical solution. The result is shown in Fig. 1.18 which illustrates how the percentages in the injection, blood, and excretion compartments vary with time.

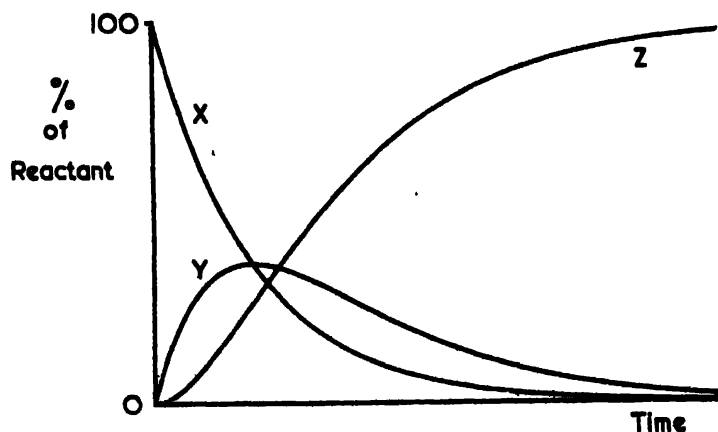


FIG. 1.18. Percentage changes in reactants with time at site of introduction (X), in blood (Y), and excreted (Z), in a three compartment system.

This result clearly accords with experience of such a problem, for the ingested substance will leave the gut at a decreasing rate. At some intermediate time, a maximum amount and concentration would be expected in the blood. The analogue computer can readily show the results when the rate constants  $k_1$  and  $k_2$  are varied and in this way the model can be matched to fit actual conditions. This example has been illustrated in greater detail by reference to the uptake and elimination of radio iodine in the thyroid gland (Ledley, 1965). The uptake curve in the thyroid resembles curve Y.

An example of a more complex compartmental system in respiratory physiology has been illustrated, using  $^{133}\text{Xenon}$  for studying regional ventilation of the lung (Matthews & Dollery, 1965).

## CONCLUSION

The present chapter is not intended to be an exhaustive report on all the applications of computers to medical problems, but rather to outline current advances. Many more investigations have been reported (see references to text-books) in neurophysiology, in gastroenterology, in simulation studies of the liver and other organs, in malignant disease, in the computation of radiation doses, and in epidemiological studies.

The potentialities for the future are obviously very considerable. Most medical and biological systems are controlled by large complexes of variables operating simultaneously. In the past, mathematical symbolism, always applicable to the physical sciences, was in general too complex for solution when applied to biology and medicine. This situation is undergoing a rapid change and problems can now be solved which would previously have deterred even the most enthusiastic research worker. Computers are stimulating new approaches to old medical problems and pointing the way to far reaching advances in medical science.

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## CHAPTER 2

# DISEASES OF MUSCLE

by

P. K. THOMAS

AN adequate classification of disorders of skeletal muscle is not at present feasible, since the basis of many of the conditions that affect muscle is unknown. The subject will therefore be considered under the separate headings of muscular dystrophy, inflammatory myopathy, metabolic and toxic myopathy, and myasthenia gravis, although it must be emphasized that this subdivision is largely a matter of descriptive convenience.

## MUSCULAR DYSTROPHY

### Classification

The muscular dystrophies can be defined as a group of genetically-determined degenerative disorders of muscle. Much confusion has arisen in the past over difficulties of classification and although it is not yet possible to produce a scheme that adequately relates the known clinical and genetic features, significant advances have been made over the past few years. At present, the most satisfactory classification is that of Walton & Nattrass (1954) as modified by Walton (1964). This recognizes the following categories, but is probably still incomplete:

1. Duchenne.
2. Limb-girdle.
3. Facio-scapulo-humeral.
4. Distal.
5. Ocular.
6. Congenital.

In addition to these examples of "pure" muscular dystrophy, Walton recognizes a second group in which there is associated myotonia:

1. Myotonia congenita.
2. Dystrophia myotonica.
3. Paramyotonia congenita.

Other important contributions to the classification of the muscular dystrophies in recent years have been made by Stevenson (1953), Chung & Morton (1959), Morton & Chung (1959) and Dubowitz (1960).

### Duchenne Type

Formerly known as pseudo-hypertrophic muscular dystrophy, this type can be subdivided into two forms, the commoner of which is inherited as a

sex-linked recessive trait. It thus characteristically affects male children, usually with an onset in the early part of childhood, but sometimes during adolescence or early adult life. Initially, the pelvic girdle muscles are involved, giving rise to the well-known difficulty in getting up from a sitting or lying position, so that the child "climbs up his legs" with his arms to gain the erect position. Later the shoulder girdle and trunk muscles are affected, followed by the more distal limb muscles. Pseudo-hypertrophy is frequent, most often involving the calves, but also the deltoid, quadriceps or other muscle groups. It is the most rapidly progressive form of muscular dystrophy, the children usually becoming incapacitated within about ten years of onset, although families with a more benign form of the disease are encountered (Dubowitz, 1960). Permanent arrest in the progress of the disease is not seen. Contractures and skeletal deformities develop in the later stages and death occurs from pneumonia, or cardiac failure consequent upon an associated cardiomyopathy (Berenbaum & Horowitz, 1956).

In the second and much rarer form of Duchenne dystrophy, there is an autosomal recessive inheritance, both sexes therefore being affected. The progress of the disorder in this form is less rapid, the clinical features being otherwise similar (Dubowitz, 1960).

### **Limb Girdle Type**

This variety is usually inherited as an autosomal recessive trait, sometimes as an autosomal dominant, but sporadic cases are quite common. Both sexes are thus affected, the onset usually occurring during adolescence or early adult life and with involvement initially of either the pelvic or shoulder girdle muscles. Pseudo-hypertrophy is seen occasionally. The rate of progress is variable, but is generally considerably slower than in the Duchenne variety, gradual involvement of both the upper and lower limb girdle, trunk and proximal limb muscles taking place. The weakness of the lower trunk and pelvic girdle muscles gives rise to a lordotic stance and "waddling" gait. The affection of muscles can be very selective, with severe atrophy of one muscle, such as brachioradialis, and preservation of the adjacent forearm muscles, a feature that may be helpful in the differential diagnosis from non-dystrophic proximal myopathies. Skeletal deformities are rare, as is cardiomyopathy. Arrested cases are encountered, particularly with localized pelvifemoral involvement, but are not common.

### **Facio-scapulo-humeral Type**

The inheritance in this variety usually shows an autosomal dominant pattern, but sometimes it may be transmitted as an autosomal recessive trait. The age of onset is variable, the symptoms beginning in childhood, adolescence or adult life and first involving the facial and shoulder girdle muscles. The facial weakness results in a myopathic facies with pouting of the lips and a "transverse" smile, difficulty in eye closure and dysarthria. Extension to the pelvic girdle and trunk then occurs and later to the more distal limb muscles, particularly in the legs. Pseudo-hypertrophy is rare, as is skeletal deformity and cardiomyopathy. The progress of the disorder is generally slow and abortive cases with slight or minimal involvement are relatively common.

### Distal Myopathy

The distal form of muscular dystrophy, although originally described by Gowers, is rare in Britain, but occurs more commonly in Sweden where it has been studied by Welander (1951). The disorder is inherited in a dominant manner and occurs more frequently in men. It usually begins between the ages of 40 and 60 and is only slowly progressive. Weakness is commonly first noticed in the small hand muscles, gradually spreading proximally, but involving the forearm extensor group before the flexors. In the lower limbs, it initially affects the distal extensor muscles. Pseudo-hypertrophy is occasionally present.

### Ocular Myopathy

This disorder was first adequately recognized as a muscular dystrophy by Kiloh & Nevin (1951), cases having formerly been described as progressive nuclear ophthalmoplegia. The initial symptoms most often become manifest during adolescence, although the condition may begin at any age from infancy to over 50. Both sexes are affected with approximately equal frequency and the transmission is by a dominant gene. Ptosis is commonly the presenting symptom, followed by involvement of the external ocular muscles. Progress is usually slow, but the disorder may advance to affect the facial and masticatory muscles, those of the neck and shoulder girdle, and sometimes also the pelvic girdle musculature. In some cases pharyngeal weakness with dysphagia occurs (Lees & Liversedge, 1962; Bray, Kaarsoo & Ross, 1965) and in others there is an associated cerebellar degeneration (Walton, 1964), a combination of a spino-cerebellar degeneration and peroneal muscular atrophy (Stephens, Hoover & Denst, 1958) or retinitis pigmentosa (Walsh, 1957).

### Congenital Myopathy

It is clear that there are many causes for the syndrome of hypotonic weakness in children and muscle biopsy is of considerable value in their differential diagnosis (Greenfield, Cornman & Shy, 1958). The term *amyotonia congenita* (Oppenheim's disease) has now been discarded. Former descriptions of this disorder probably included a variety of conditions. The term *benign congenital hypotonia* was introduced by Walton (1957) to describe cases of hypotonic weakness in childhood dating from birth, some of whom recovered completely, others of whom showed persisting non-progressive muscular disability throughout life. It would seem most satisfactory to confine the term to the group in which recovery occurs. The nature of the underlying disturbance in these cases is unknown. Some examples display muscle fibres of uniformly small diameter.

Although occasional cases of Duchenne dystrophy or dystrophia myotonica have symptoms dating from birth, other congenital myopathies of a non-progressive type are encountered. The categorization of such conditions is as yet incomplete. The most fully documented example is *central core disease*, initially recognized by Shy & Magee (1956) and studied in greater detail by Engel and co-workers (1961). Patients with this condition show non-progressive proximal muscle weakness throughout life. The affected muscles are of reduced bulk and there may be some depression of the tendon reflexes.

In the family described by Shy & Magee, the disorder was probably inherited as an incompletely dominant trait. Histologically, the muscle fibres display a central core with abnormal staining properties. In electron microscope preparations, mitochondria are almost completely absent in this region, the myofibrils are more closely packed and the regular alignment of the Z lines is disturbed.

A further type of non-progressive proximal congenital myopathy has been reported by Shy and co-workers (1963) under the title of *nemaline myopathy* and reviewed more recently by Hopkins, Lindsey & Ford (1966). The distinctive feature is the presence of collections of rod-shaped structures, particularly in the subsarcolemmal region of the fibres. When examined with the electron microscope, they show a 145 Å periodicity and are possibly chemically related to myosin. The histological features of both central core disease and nemaline myopathy have been reported in the same individual in a family with nemaline myopathy (Afifi, Smith & Zellweger, 1965), thus raising questions about the specificity of these morphological changes.

Recently, another congenital muscle disorder has been described by Spiro, Shy & Gonatas (1966) and termed *myotubular myopathy*. In this, a progressive myopathy dating back to early infancy and involving the external ocular, facial and limb muscles was found to be associated with the presence in the muscles of abnormal fibres (myotubes) of a type normally observed only in early foetal life. The central portion of such fibres contains nuclei and mitochondria but is devoid of myofibrils.

The congenital occurrence of skeletal deformities associated with fixation of joints but without bony ankylosis is known as *arthrogryposis multiplex congenita*. Although this syndrome may arise from a variety of causes, some cases are the result of congenital myopathy, the deformities being secondary to fibrotic contracture (Banker, Victor & Adams, 1957; Pearson & Fowler, 1963).

### Myotonic Syndromes

The phenomenon of myotonia as observed clinically consists of a delay in relaxation after a sustained or forcible muscular contraction. Striking an affected muscle with a tendon hammer may produce a persisting localized contraction (percussion myotonia). Electromyographic observations on myotonia in goats and in man have demonstrated that it is associated with repetitive high frequency discharges of single muscle fibres or groups of muscle fibres which persist after nerve blocking or section, or curarization, indicating a primary disturbance of muscle.

In *myotonia congenita* (Thomsen's disease), myotonia is evident throughout life, but tends gradually to diminish. The disorder is inherited as an autosomal dominant trait. Generalized muscular hypertrophy is sometimes present. Occasionally restricted muscle weakness and wasting are detectable. The myotonia is usually diminished by repeated use of the muscles.

In *dystrophia myotonica*, in addition to myotonia, muscle weakness and wasting regularly occur; this predominantly affects the face, the sternomastoids and the distal limb muscles. The myotonia is most evident in the small hand muscles, the forearms and the tongue. Weakness of the respiratory muscles, including the diaphragm, may lead to chronic hypoventilation and

somnolence. Abnormalities of the electrocardiogram are frequently present, with evidence of defects in the conducting system and cardiomyopathy (Payne & Greenfield, 1963; Lee & Hughes, 1964). Paroxysmal disorders of rhythm may occasionally occur (Kohn, Faires & Rodman, 1964), but cardiovascular symptoms are otherwise unusual (Church, 1967). Recent observations have also drawn attention to abnormalities of smooth muscle. Dysphagia from oesophageal involvement is the most common symptom (Fischer *et al.*, 1965; Pierce, Creamer & MacDermot, 1965). Disturbances of bowel function also occur (Kohn, Faires & Rodman, 1964), giving rise to constipation or diarrhoea, and steatorrhoea has been reported (Kaufman & Heckert, 1954). The occurrence of cataracts is common, as is gonadal atrophy and, in men, frontal baldness. There may be an associated mental defect. Endocrine studies (Marshall, 1959; Caughey & Saucier, 1962) show a reduced urinary excretion of 17—oxosteroids related to deficient androgen production. The urinary gonadotrophin excretion is variable but may be elevated, indicating that the hypogonadism is not of pituitary origin. Thyroid function is also sometimes impaired. Although the histological features of the testicular atrophy resemble those seen in Klinefelter's syndrome, the nuclear sex chromatin pattern is normal (Marshall & Thomas, 1958). Radiological abnormalities of the skull have been described, including increased thickness of the cranial vault, hyperostosis interna, a reduction in size of the pituitary fossa and extensive frontal sinuses (Caughey, 1952).

The age of onset of dystrophia myotonica is variable, the disease beginning most often during adolescence or early adult life, but it may commence in childhood or later in adult life. Progression is usually slow. In some patients, weakness may not be obtrusive in the early stages of the disease, the myotonia predominating; in others, the myotonia may not be clinically obvious, being only detectable electromyographically (Ziegler & Rogoff, 1956). These variations in the clinical manifestations, together with the occasional occurrence of dystrophic changes in myotonia congenita, have led to the suggestion that dystrophia myotonica and myotonia congenita are variants of the same disorder (Maas & Paterson, 1950). The currently held opinion, however, is that despite the uncertainty as to the genetic basis, the two conditions are usually separable clinically. The distinction is important, as the prognosis differs greatly.

Dystrophia myotonica is inherited as an autosomal dominant trait. It has often been claimed that the condition exhibits "anticipation", such that in one generation only cataract, for example, may occur and in the next the other manifestations appear. It seems probable that this is a spurious observation resulting from case selection in a disease that shows a highly variable age of onset with a much greater correlation as to age of onset between siblings than between parents and offspring (Penrose, 1947).

The nosological status of *paramyotonia* is the subject of debate, it being variously considered to be a separate disease entity (Baxter & Dyck, 1961; Magee, 1963), to be identical with hyperkalaemic familial periodic paralysis (adynamia episodica hereditaria), which is the view proposed by Drager, Hammill & Shy (1958), or that it is merely a variant of myotonia congenita. It is inherited as an autosomal dominant trait. As originally defined by Eulenberg, the condition consists of myotonia that appears on exposure to

cold and which may be associated with attacks of muscular weakness. In addition, the myotonia may be aggravated rather than relieved by repeated use of the muscle (myotonia paradoxa) as emphasized by Magee (1963) and sometimes persisting weakness and wasting, usually in the legs, is seen (Hudson, 1963). It is the combination of myotonia and episodic muscular weakness that has suggested an affinity or identity with hyperkalæmic familial periodic paralysis. However, until the metabolic basis of these disorders is further understood, it will be difficult to assess the significance of such clinical associations.

**Treatment of Myotonia.** Quinine, procaine amide and corticosteroids are all effective in reducing myotonia. Their relative merits were assessed by Leyburn & Walton (1959). Quinine was found to be the least effective preparation. Prednisone and procaine amide were approximately equally beneficial. However, the long-term use of corticosteroids is likely to give rise to troublesome side-effects and with procaine amide, there is a small risk of agranulocytosis. A recent study (Munsat, 1967) has suggested that diphenylhydantoin is as effective as procaine amide.

#### **Laboratory Investigations in Muscular Dystrophy**

**Serum Enzymes.** The serum level of a number of cellular enzymes may be elevated; this is probably the result of leakage from the affected muscle fibres rather than any specific metabolic defect. Of the enzymes so far studied, the elevation is greatest for creatine kinase, although others such as aldolase and aspartate and alanine transaminase are also increased. The elevation is most marked in early cases of Duchenne dystrophy and may be apparent before there is clinical evidence of disease, tending to fall in the later atrophic stages (Dreyfus & Schapira, 1962; Hughes, 1963; Pearce, Pennington & Walton, 1964a). Raised levels may also be encountered in patients with the limb girdle and facio-scapulo-humeral forms, and in dystrophia myotonica, but with less constancy than in the Duchenne variety.

**Electromyography.** Electromyographic examination is helpful in diagnosis, particularly in the differentiation from conditions producing denervation atrophy of muscles. As with other disorders in which diffuse loss or destruction of muscle fibres occurs, abnormalities may be detected in the motor unit potentials. These tend to be reduced in amplitude and duration and to be abnormally polyphasic. In contrast to the findings in a denervation atrophy, the number of units active during a maximal voluntary contraction may not be markedly reduced until the later stages of the disease. Myotonia, when present, is recognized by the occurrence of high frequency repetitive discharges. These may follow active muscular contraction or can be provoked by needle movement or percussion of the muscle adjacent to the needle. A recent account of the electromyographic changes in muscle disease has been given by Richardson (1964).

**Muscle Biopsy.** In performing a muscle biopsy, it is important to choose a moderately affected muscle. If a muscle that is only mildly affected is chosen, the changes may not be definite enough for diagnostic purposes and if the muscle is too severely involved, few surviving fibres may be present, again making diagnosis difficult. In patients with the progressive dystrophies, variation in the size of the muscle fibres is one of the most striking findings,



some being abnormally large and others small and atrophic. The number of fibres present shows a progressive reduction with advance of the disease; at the same time, the amount of endomysial connective tissue tends to increase and to show fatty infiltration, especially in a pseudo-hypertrophic muscle. Degenerative changes of various kinds may be seen in the fibres and at times necrosis with associated phagocytosis is observed. Central migration of the sarcolemmal nuclei occurs, and longitudinal chains of centrally placed nuclei are particularly characteristic of dystrophia myotonica. Also especially common in dystrophia myotonica is the occurrence of fibres in which the peripheral myofibrils have a circular rather than a longitudinal disposition (striated annulets) or the peripheral sarcoplasm is devoid of myofibrils (sarcoplasmic masses). The salient histological features in the rare congenital myopathies, central core disease, nemaline myopathy and myotubular myopathy have already been discussed.

**Detection of Carriers.** An elevation of the serum creatine kinase activity is detectable in a high proportion of female carriers of the sex-linked recessive gene producing Duchenne-type dystrophy (see Pearce, Pennington & Walton, 1964b) and is thus helpful in genetic counselling. Minor electromyographic changes in such female carriers have been claimed (van den Bosch, 1963) and slight abnormalities may also be detected clinically (Emery, 1963) and in muscle biopsies (Dubowitz, 1963, Pearce, Pearce & Walton, 1966). A possible explanation of these findings is that in some cells of female carriers, one X-chromosome is inactivated, permitting the abnormal gene carried by the other X-chromosome to produce dystrophic changes (Lyon, 1961).

## INFLAMMATORY MYOPATHY

The term *inflammatory myopathy* has been employed to group together a number of conditions in which the muscles show histological evidence of non-suppurative inflammatory changes which may or may not be associated with degeneration or destruction of muscle fibres. The more frequent use of diagnostic muscle biopsy in recent years has led to the increased recognition of such cases, although much uncertainty still exists as to their nature and classification.

### Polymyositis and Dermatomyositis

Reviews of the clinical and pathological features of polymyositis have been made by Walton & Adams (1958), Barwick & Walton (1963), Pearson (1964a) and Rose & Walton (1966). This disorder may begin in childhood or at any age during adult life. Although the onset is usually gradual, acute cases may arise, sometimes following an infective illness or sulphonamide administration. The course of the illness is often fluctuating, with spontaneous remissions and exacerbations, but at other times is steadily progressive. Spontaneous regression and arrest may occur. The limb girdle and proximal limb muscles are most commonly affected. In severe cases there may be widespread weakness and wasting in the limbs and trunk, together with involvement of the neck and bulbar muscles. Particularly in the acute cases, muscle pain and tenderness are often noticeable, but are not constantly present; there may be a constitutional upset with fever and malaise.

and myoglobinuria may occur. In chronic cases, especially in children, fibrous contractures may develop. Calcification of the muscle and subcutaneous tissues is occasionally seen, again particularly in children (myositis ossificans) and ulceration of calcified deposits through the skin may take place.

In a proportion of cases, there are accompanying skin changes. The classical skin lesions of dermatomyositis, which according to Pearson (1964a) are present in approximately 40 per cent of cases of polymyositis, consist of a mottled, scaling, erythematous rash, often with a violaceous colouration, and seen predominantly over the face and neck, the upper part of the trunk and the extensor aspects of the forearms and hands. Photosensitivity is quite common. In acute cases, the skin and subcutaneous tissues may be oedematous. Other cases show changes of sclerodermatous type, most frequently over the face, forearms and hands, or atrophic skin lesions with vitiligo, pigmentation and telangiectasia.

Raynaud's phenomenon occurs in many patients, as do articular manifestations similar to those of rheumatoid arthritis. These are usually mild (Pearson, 1959). Visceral involvement is also occasionally encountered, including hypotonicity of the oesophagus (Donoghue, Winkelman & Moersch, 1960). Disturbances of the small intestine of the kind observed in scleroderma have been found and a mild malabsorption syndrome may result. Minor electrocardiographic abnormalities have also been reported.

**Laboratory Findings.** The erythrocyte sedimentation rate may be raised, but is often normal in chronic cases. Abnormalities of the serum proteins also occur in a proportion, with elevation of the gamma globulin fraction (Gavrilescu & Small, 1962). An elevation of a variety of serum enzymes, including creatine kinase, aldolase and to a lesser extent, aspartate and alanine transaminase, is frequently observed in acute and subacute cases (Rose & Walton, 1966), but is less constant when the disease is of long standing.

Electromyographic examination reveals myopathic abnormalities in the motor unit potentials. About half the cases also show evidence of abnormal excitability of the muscle fibres. The electrical activity evoked by needle movement may be increased and spontaneous discharges from single muscle fibres or groups of fibres (fibrillation) may be observed. These changes are normally indicative of denervation and when they were originally found, they led to use of the term *neuromyositis*. It is possible, however, that they result from segmental necrosis of muscle fibres so that portions become isolated from the end-plate region. Damage to the terminal intramuscular branches of the nerve fibres has also been suggested as an explanation and histological evidence of involvement of the peripheral nerves is sometimes found (McEntee & Mancall, 1965; Banker & Victor, 1966).

Muscle biopsies reveal both destruction and regeneration of muscle fibres, a variable infiltration of the connective tissues with inflammatory cells, and endomysial fibrosis. Some cases that are otherwise clinically typical show no inflammatory changes, but as the involvement of muscles is often patchy, sampling difficulties arise in the taking of biopsy specimens. In children with dermatomyositis, vascular changes are prominent not only in muscle but also in other tissues (Banker & Victor, 1966). Denny-Brown (1960) considered that a diagnosis of polymyositis should not be made unless

inflammatory changes are demonstrable histologically, but a response to treatment with corticosteroids may be seen in cases in which muscle biopsy is negative (Rose & Walton, 1966). In late "burnt-out" cases, the appearances in muscle biopsies may be difficult to distinguish from those of muscular dystrophy (Christensen & Rossel, 1964).

#### **Association between Polymyositis and the "Collagen Diseases"**

As already discussed, many patients with polymyositis show mild abnormalities suggesting affinities with the collagen diseases, such as features of scleroderma or arthropathy of rheumatoid type. Circulating rheumatoid factor was detected in about 50 per cent of cases by Pearson (1959), but in a much smaller proportion by Barwick & Walton (1963). Conversely, a polymyositic syndrome may accompany systemic lupus erythematosus, rheumatoid arthritis and scleroderma (Walton & Adams, 1958; Barwick & Walton, 1963; Rose & Walton, 1966) and also Sjögren's syndrome (Bunim, 1961; Shy, 1962; Pearson, 1964a).

Patients with the collagen disorders may also show muscle lesions distinct from the syndrome of polymyositis. In cases of rheumatic fever, rheumatoid arthritis, systemic lupus erythematosus, polyarteritis nodosa and scleroderma, multiple focal collections of inflammatory cells are often observed in the muscles without any accompanying clinical disturbance. This *focal nodular myositis* corresponds to the nodules originally recognized in cardiac muscle by Aschoff in rheumatic fever. Pearson & Yamazaki (1958) have described an unusual vacuolar myopathy in occasional cases of systemic lupus erythematosus, again without any detectable clinical accompaniment, although the possibility of this being caused by the administration of chloroquine has to be considered in such cases (Rewcastle & Humphrey, 1965).

Muscle pain and tenderness are not uncommon in polyarteritis nodosa. Histological examination of the muscles in this disorder, apart from denervation atrophy secondary to the peripheral nerve lesions, may show focal infarction or hæmorrhage, or extension into the surrounding muscle of inflammation in the wall of an intramuscular artery.

#### **Carcinomatous Myopathy**

Clinically typical dermatomyositis or, less commonly, a proximal myopathy, may be encountered in patients with carcinoma, most frequently of the breast, gastro-intestinal tract, bronchus or ovary (Williams, 1959; Rowland & Schotland, 1965). These disorders may also be seen in association with malignant reticuloses. As with other non-metastatic complications of malignant disease, the occurrence of muscle disease may antedate the discovery of the tumour. In men over the age of 60, the onset of proximal muscle weakness is highly likely to be due to a carcinoma, the possibility being considerably less in women (Shy & Silverstein, 1965). Patients with non-metastatic neurological complications of carcinoma, such as cerebellar degeneration or sensory neuropathy, may also show associated muscle disorder of polymyositic type not attributable to denervation (Denny-Brown, 1948) and the term *carcinomatous neuromyopathy* has been applied to such cases (Brain & Henson, 1958). It has also been adopted by Shy & Silverstein (1965), who reported that evidence of primary muscle disease and denerva-

tion atrophy frequently coexist in muscle biopsies taken from patients with proximal muscle weakness related to carcinoma.

### **Ætiology of Polymyositis**

The cause of polymyositis is not yet known. The presence of inflammatory cells in the muscles and the fact that it is commonly accompanied by features of the collagen disorders have suggested an auto-immune mechanism. Similarly, the association with carcinoma has raised the possibility that hypersensitivity to an antigen derived from the tumour is involved. The syndrome may represent a hypersensitivity response to a number of different antigens. As defined at present, however, it may include other types of muscle damage not yet recognized, and for this reason, Shy (1962) has objected to the tendency to employ the term polymyositis too readily to cases of late onset myopathy.

### **Treatment**

Treatment with corticosteroids is advisable in most cases, usually with an initially high dosage, followed by a maintenance dose regulated by the clinical response and the serum enzyme levels (Barwick & Walton, 1963; Pearson, 1964a). The response is frequently good in acute or subacute cases in adults, but less satisfactory in chronic cases. When associated with carcinoma or with Sjögren's syndrome, no more than an initial temporary improvement is to be expected. The response to corticosteroids in children has been reported as being less satisfactory (Wedgewood, Cook & Cohen, 1953), although Rose & Walton (1966), on the other hand, found that the prognosis was relatively better in the younger age groups.

### **Myopathy in Sarcoidosis**

Sporadic sarcoid nodules are found in random muscle biopsies in 50 per cent or more of patients with sarcoidosis (Wallace *et al.*, 1958), without any clinically detectable abnormality of the muscles except for slight muscle discomfort in a few instances. Occasional cases have been reported who have shown a predominantly proximal myopathy with diffuse sarcoid infiltration of the affected muscles, but no evidence of sarcoidosis elsewhere (Crompton & MacDermot, 1961; Hinterbuchner & Hinterbuchner, 1964). Treatment with corticosteroids has been without benefit.

### **Polymyalgia Rheumatica**

Polymyalgia rheumatica is a disease occurring mainly in late life characterized by diffuse muscular and periarticular pain, most severe around the shoulders and hips (Gordon, 1960). Pain and stiffness may be sufficiently troublesome to cause virtual incapacitation, but there is no muscular weakness. There is often a low-grade pyrexia, a mild normochromic anæmia and an eosinophilia. The sedimentation rate is constantly raised, the elevation being considerable, and increased levels of plasma fibrinogen and alpha-2 globulin are frequently found. The Rose-Waaler test is negative.

The disorder has been considered to represent a hypersensitivity response in the periarticular and intramuscular connective tissue. However, no very definite histological changes have been discovered in muscle biopsies.

Arterial biopsies, on the other hand, have shown evidence of giant-cell arteritis (Alestig & Barr, 1963; Hamrin, Johnsson & Landberg, 1964). The usual clinical manifestations of temporal arteritis do not occur. The disease responds dramatically to corticosteroid treatment.

## **METABOLIC AND TOXIC MYOPATHY**

### **Hypokalaemic and Hyperkalaemic Paralysis**

The muscle membrane is normally electrically polarized, the interior of the fibre being approximately 90 mV negative to the exterior. This is related to the unequal distribution of potassium ions across the membrane, there being a high intracellular and a low extracellular concentration. The muscle action potential involves depolarization associated with an efflux of potassium ions. The membrane potential is therefore affected by alterations in the intracellular and extracellular concentrations of potassium, hyperkalaemia giving rise to a reduction in the size of the resting potential (partial depolarization) and hypokalaemia producing an elevation (hyperpolarization). If of sufficient magnitude, both will lead to the muscle fibre becoming inexcitable. With partial depolarization, the membrane may in fact be hyperexcitable, resulting in spontaneous or repetitive discharges of muscle fibres. Weakness is usually seen at plasma potassium levels of less than 2.5 mEq per litre or greater than 7 mEq per litre and flaccid paralysis at levels of 2 mEq per litre and 9 mEq per litre. The paralysis tends to spare the muscles innervated by the cranial nerves, affecting those of the limbs and trunk. Electrocardiographic changes also occur.

Episodic muscular weakness resembling the attacks of familial periodic paralysis may be seen in primary aldosteronism (Conn, 1955). This is usually the result of an aldosterone secreting adrenal tumour giving rise to hypokalaemia from excessive urinary loss of potassium. The attacks may be accompanied by tetany and peripheral paræsthesiæ. Hypokalaemic weakness may also arise from excessive potassium loss because of renal tubular defects or from the gastro-intestinal tract, in the recovery phase of severe diabetic ketosis, and as a complication of treatment with chlorothiazide and other diuretic agents. Hyperkalaemic weakness may follow potassium retention in renal failure and may occur during diabetic ketosis.

### **Familial Periodic Paralysis**

The role of potassium in familial periodic paralysis was first established in 1934, but observations over the past 15 years have made it apparent that there are three forms of this disorder. Apart from patients in whom the attacks are associated with hypokalaemia, there are some in whom the potassium level is raised (adynamia episodica hereditaria; Gamstorp, 1956) and others in whom it is unaltered. Considerations of treatment clearly make it important to establish the particular metabolic disturbance in any new case. On the other hand, the situation is complicated by the fact that occasional individuals in clinically similar attacks may at varying times show reduced, normal or elevated potassium levels (Pearson, 1964b).

**Hypokalaemic Familial Periodic Paralysis** is the most commonly encountered form of this rare syndrome. It is inherited as an autosomal

dominant trait, and the manifestations are more severe in males. Attacks of paralysis most often begin in early adult life, but become less frequent or cease in later life. They tend to involve the limb and trunk muscles, sparing the respiratory muscles and those innervated by the cranial nerves. The average duration is six to twelve hours, but they may persist for as long as one or two days. They are frequently provoked by prolonged rest after exertion or by a large carbohydrate meal. In the attacks, there is flaccid paralysis and loss of the tendon reflexes; the muscles are inexcitable to direct electrical stimulation and the electrocardiogram may be abnormal. In the later stages of the disease, permanent weakness and wasting, especially of the pelvic girdle and lower limb muscles, may develop.

The plasma potassium level characteristically falls in attacks, but paralysis may appear at levels within the normal range and factors other than simple hypokalaemia are therefore involved. There is a reduction in the urinary excretion of potassium, potassium migrating into the muscles (Grob, Liljestrand & Johns, 1957). Water also probably enters, as electron microscopy demonstrates a greatly dilated sarcoplasmic reticulum (Shy *et al.*, 1961). It was suggested (McArdle, 1956) that this is the result of a partial metabolic block in the breakdown or synthesis of carbohydrate, leading to the accumulation of indiffusible intermediary substances, but this has not been substantiated (Engel, Potter & Rosevear, 1967). The precise mechanism of the paralysis is not understood. It does not appear to be due to hyperpolarization of the muscle membrane (Shy *et al.*, 1961; Creutzfeldt *et al.*, 1963). Claims have been made (Conn & Streeten, 1960) that sodium is retained in the attacks and that there is an increased excretion of aldosterone, but this has not been confirmed.

Diagnosis is achieved by the finding of a reduced plasma potassium level in an attack. It may be necessary to provoke an attack by the administration of glucose and insulin. In treating the disorder, individual attacks respond to oral potassium and their frequency may be lessened by a low carbohydrate, high potassium diet. Spironolactone has also been suggested as a prophylactic measure (Poskanzer & Kerr, 1961a).

The attacks in **Adynamia Episodica Hereditaria (Hyperkalaemic Periodic Paralysis)** are usually more frequent and of shorter duration than in the hypokalaemic form and may begin during childhood. They are provoked by rest after exercise, but not by carbohydrate meals. Electromyographic examination reveals muscle fibre hyperexcitability with myotonic phenomena, succeeded by loss of function (Buchthal, Engbaek & Gamstorp, 1958). Between attacks, percussion myotonia may occasionally be observed in the tongue and thenar muscles and myotonic "lid-lag" noted on looking down after upward gaze for a few seconds, but clinical myotonia is not a prominent feature of the condition. Persisting muscle weakness and wasting may develop and are more common than in the hypokalaemic variety. The disorder is inherited as an autosomal dominant trait.

The plasma potassium level is usually raised in a severe attack, but weakness may occur with values within the normal range. The urinary excretion of potassium is increased, probably because of leakage from muscle cells (McArdle, 1962). The muscle membrane potential is reduced both between attacks and to a greater extent during attacks (Creutzfeldt *et al.*,

1963). The depolarization of the muscle membrane may well be responsible for the weakness and the hyperexcitability of the muscle fibres found electromyographically, but it is as yet uncertain how it is related to the myotonia that is observed clinically. The full explanation of the depolarization is not known. It is clear that it cannot be related directly to the hyperkalæmia.

For diagnostic purposes, if an attack cannot be precipitated by rest after exertion, potassium administration is employed. Treatment is largely of a prophylactic nature with diuretics to induce potassium loss, either using hydrochlorothiazide or the carbonic anhydrase inhibitor dichlorphenamide (McArdle, 1962).

**Normokalæmic Periodic Paralysis.** A third type of periodic paralysis has been reported by Poskanzer and Kerr (1961b) with clinical features similar to those of the hyperkalæmic variety except that the attacks are of longer duration. The plasma potassium is normal in the episodes, the weakness being aggravated by potassium administration but improved by sodium chloride. A combination of acetazolamide and 9- $\alpha$ -fluorohydrocortisone is helpful in prophylaxis.

#### **Hereditary Myophosphorylase Deficiency (McArdle's Syndrome)**

This is a rare disorder due to a single completely recessive autosomal gene (Schmid & Hammaker, 1961) and was initially described by McArdle (1951). The onset of symptoms is in childhood, exercise producing painful muscle cramps. In some patients, exercise may be followed by transient myoglobinuria and limb girdle weakness may appear in later life. Cases of myophosphorylase deficiency of late onset have also been reported (Engel, Eyerman & Williams, 1963).

Electromyography of muscle affected by a cramp shows that the shortening is unaccompanied by propagated muscle action potentials and is therefore a physiological contracture. Exercise, particularly ischæmic exercise, fails to produce the normal elevation of blood lactate and pyruvate, indicating a block in muscle glycogen breakdown. Muscle phosphorylase, which is necessary for the initial stage of this process, has been shown to be absent or grossly deficient. The muscle fibres contain excessive amounts of glycogen, mainly in the interfibrillary space of the I band and beneath the sarcolemma (Schotland *et al.*, 1965). The deficiency is confined to muscle glycogen breakdown, hepatic glycogenolysis being unaffected. Ingestion of glucose or fructose prior to activity sometimes increases exercise tolerance.

The disorder has been reviewed recently by McArdle (1964) and Rowland and co-workers (1966). It can be considered as one form (Cori type V) of the glycogenoses. Skeletal muscle is also involved in  $\alpha$ -glucosidase deficiency (Pompe's disease, Cori type II) and in amylo-1,6-glucosidase deficiency (Forbes' disease, Cori type III), but in these forms muscle symptoms are not prominent.

#### **Hereditary Deficiency of Muscle Phosphofructokinase**

A new type of glycogenosis has recently been described (Tarui *et al.*, 1965), which appears to be inherited as an autosomal recessive trait. The affected individuals complain of life-long exercise intolerance, vigorous or prolonged activity giving rise to muscle weakness and stiffness. On ischæmic

exercise, venous lactate fails to increase. The muscles show a considerable accumulation of hexose monophosphates and a moderate increase in glycogen content. Muscle phosphofructokinase was shown to be almost totally absent, whereas red cell phosphofructokinase was only partially affected.

### **Myoglobinuria**

Myoglobin may appear in the urine in a variety of disturbances involving muscle, including crush injuries to the limbs or extensive infarction from arterial occlusion; damage by electric shock; acute polymyositis; in Haff disease, epidemics of which have occurred in eastern Europe possibly caused by an unidentified toxic substance present in contaminated fish; and by the venom of a sea-snake (Reid, 1961). It is also occasionally encountered in normal subjects after severe exertion (Greenberg & Arneson, 1967). Its occurrence in some cases of hereditary absence of myophosphorylase (McArdle's syndrome) has already been mentioned. In severe cases of myoglobinuria, acute renal failure may result.

**Idiopathic Paroxysmal Myoglobinuria** is a rare disorder, sometimes with a family history of the same condition, characterized by recurrent attacks of muscle pain and weakness, associated with myoglobinuria. Occasionally, permanent muscle wasting and weakness gradually develop as a sequel to repeated attacks. The disease has been separated into two types (Korein, Coddon & Mowry, 1959). The first usually begins in adolescence or during early adult life, the attacks being provoked by exercise. The second type occurs most commonly in children, the episodes tending to follow infections. Apart from myoglobin, the serum concentration of other intracellular substances such as creatine and muscle enzymes is increased. Muscle biopsies reveal widespread swelling and fragmentation of fibres. The suggestion that an abnormal myoglobin is responsible has not been confirmed. It seems more likely (Pearson, Beck & Bland, 1957) that the myoglobin is released in a non-specific manner, together with other intracellular components, because of a destructive lesion of the fibres. Rapid regeneration normally ensues. The nature of the underlying disorder of function has been uncertain, but recently Larsson and co-workers (1964) have described a number of families with a myopathy that resembles the first type of idiopathic paroxysmal myoglobinuria as defined by Korein *et al.* (1959). The disease, which begins in childhood, appears to show a monohybrid autosomal recessive mode of inheritance, and is manifested as a reduced capacity for physical performance, exercise producing muscle pain, cramp and weakness, sometimes associated with myoglobinuria. Muscle biopsies showed the type of changes already described. During exercise, utilization of oxygen by the active muscles is low and the elevation of lactate and pyruvate in the blood excessive, indicating abnormal glycolysis. The exact nature of the metabolic defect was not established, but it was assumed that abnormally large quantities of acid metabolites are produced, leading to damage of some muscle fibres with consequent release of their contents.

### **Myopathy Associated with Osteomalacia**

Patients with osteomalacia due to intestinal malabsorption or renal causes may develop limb girdle weakness, predominantly involving the pelvic



girdle and giving rise to a "waddling" gait. Electromyography may reveal myopathic changes and muscle biopsy show scattered atrophic fibres. The condition is not closely related to the level of ionized serum calcium and as it may be improved by vitamin D administration, a disturbance in the metabolism of this vitamin has been suggested as the basis of the disorder. Vitamin D is known to be localized at the muscle membrane (Kodicek, 1963), and may influence the transfer of calcium ions across the membrane. The strength of muscular contraction is related to the quantity of calcium entering the fibres per contraction (Bianchi & Shanes, 1959). The subject has been discussed recently by Prineas, Mason & Henson (1965).

#### **Myopathy with Mitochondrial Abnormalities**

The existence of two previously unrecognized genetically-determined childhood myopathies has recently been claimed by Shy, Gonatas & Perez (1966), in both of which mitochondrial abnormalities were observed. In one, termed *megaconial myopathy*, affected individuals displayed a slowly progressive proximal weakness. Electron microscopy of muscle biopsies demonstrated giant mitochondria which contained various types of inclusions. It was considered that in this condition there was possibly a difficulty in handling intra-cellular lipids. In the second disorder, termed *pleoconial myopathy*, proximal weakness was accompanied by attacks of flaccid paralysis and a craving for salt. Electron microscopy revealed increased numbers of mitochondria, only slightly greater than normal in size. A disturbance in the intracellular transport of cations was thought to be involved.

#### **Chloroquine Myopathy**

Muscle damage from chemical agents is uncommon, but a reversible myopathy due to the prolonged administration of chloroquine has been recognized in recent years (Whisnant *et al.*, 1963). As with many other myopathies, the proximal muscles are predominantly affected, especially those of the lower limbs. The muscle fibres show a prominent vacuolar change, resulting from the dilatation of the longitudinal component of the sarcoplasmic reticulum (Rewcastle & Humphrey, 1965). The vacuoles are associated with glycogen accumulation, suggesting an interference with muscle carbohydrate metabolism (Eadie & Ferrier, 1966). In experimental chloroquine myopathy, a selective involvement of "red" muscle fibres has been observed (Smith & O'Grady, 1966) which raises the possibility that chloroquine is bound by myoglobin.

#### **Alcoholic Myopathy**

Hed *et al.* (1962) drew attention to the occurrence of an acute myopathic syndrome encountered in chronic alcoholic subjects following a period of excessively high alcohol intake. Severe muscular aching and tenderness are the predominant symptoms and oedema of the muscles and subcutaneous tissues may be observed on examination. Slight myoglobinuria is occasionally present. Muscle biopsies reveal degeneration and necrosis of muscle fibres and serum enzyme estimations suggest that necrosis of liver cells also occurs. After ischemic exercise, the blood lactate level fails to rise (Perkoff, Hardy & Velez-Garcia, 1966), suggesting defective muscle glycogenolysis. Some of the

affected patients have in addition had an alcoholic neuropathy, but thiamine deficiency is not considered to be the cause of the myopathy.

Another syndrome has also been defined in chronic alcoholic subjects (Ekblom *et al.*, 1964) in which proximal weakness gradually develops. On electromyographic examination, myopathic changes are found and muscle biopsies demonstrate evidence of recent or previous muscle fibre destruction. On cessation of alcohol consumption, the weakness recovers.

## ENDOCRINE MYOPATHY

### Muscle Disorders Associated with Hyperthyroidism

**Chronic Thyrotoxic Myopathy** was formerly considered to be a rare disorder, but recent studies have demonstrated that this is not the case. In an unselected series of patients with thyrotoxicosis, Havard and co-workers (1963) found that muscle weakness was present in 80 per cent. Although unnoticed by many of the patients, it was the main complaint in 6 per cent. Myopathic changes in the electromyogram were evident in 88 per cent and were severe in 16 per cent. Similar electromyographic findings were obtained by Ramsay (1965). The limb girdle muscles are predominantly involved and may exhibit wasting out of proportion to the generalized loss of muscle bulk. Whether serum enzyme abnormalities occur has not yet been established. In two patients with thyrotoxic myopathy reported by Pearce, Pennington & Walton (1964a), normal values were obtained. The explanation of the myopathy is uncertain; the histological findings in muscle biopsies are usually unimpressive. A disturbance of oxidative phosphorylation has been postulated (Ramsay, 1966). Complete recovery always occurs with treatment of the thyrotoxicosis. An acute thyrotoxic myopathy used to be recognized, but its existence as a separate entity is dubious. It seems possible that such cases were instances where there was an associated weakness of the bulbar muscles (Ramsay, 1966).

The salient features in **endocrine exophthalmos (exophthalmic ophthalmoplegia)** are proptosis and diplopia. The proptosis, which may be unilateral but is usually bilateral, results from an increase in the volume of the orbital contents, including that of the external ocular muscles. Oedema of the conjunctivæ and the lids may occur and lead to corneal ulceration (malignant exophthalmos). The diplopia is secondary to weakness of the external ocular muscles. Elevation and abduction of the eyes are the movements most commonly affected, but the disorder may progress to a complete external ophthalmoplegia. The muscles are macroscopically enlarged and histologically appear oedematous, with accompanying fibrosis, fatty infiltration and collections of lymphocytes. Hyperthyroidism is present in about half of the cases and treatment of the thyrotoxicosis may aggravate the exophthalmos. In other examples, the condition appears when the patient is euthyroid or even hypothyroid subsequent to treatment of preceding thyrotoxicosis, sometimes after an interval of some years. The onset may be gradual or rapid, but spontaneous regression usually occurs ultimately (Brain, 1959).

The cause of endocrine exophthalmos is obscure. As it does not occur in hyperthyroidism due to toxic adenomas or thyroid administration, it is presumably not directly related to thyroid overactivity. It has been suggested

that it is due to thyroid stimulating hormone (TSH), but this is probably not the responsible agent since exophthalmos is rare in primary myxœdema, in which TSH levels are elevated. Exophthalmos also does not seem to be closely correlated with long-acting thyroid stimulator (Major & Monroe, 1962). An exophthalmos producing factor (EPF), assayed biologically, has been isolated from pituitary extracts and shown to be distinct from TSH (Dobyns & Steelman, 1953). An exophthalmos producing substance can also be detected in the serum of patients with exophthalmos in amounts that correlate moderately well with the clinical state (Dobyns & Wilson, 1954; der Kinderen, Houtstralanz & Schwarz, 1960), but the origin of this substance is uncertain. Evidence as to participation of the pituitary is conflicting, for although hypophysectomy may be followed by improvement, in one case pituitary ablation for unilateral exophthalmos was later followed by the development of bilateral eye signs (Furth *et al.*, 1962). Medical treatment appears to have little influence. If the condition is associated with hypothyroidism, thyroxine may produce some improvement. Other measures that have been suggested are X-irradiation of the posterior orbital tissues and corticosteroids, but these are of uncertain value. Tarsorrhaphy may be required to protect the eye or orbital decompression become necessary if there is gross proptosis.

**Periodic Paralysis.** The occurrence of attacks of periodic paralysis in patients with hyperthyroidism, although an uncommon event, has been well documented, most of the reports having appeared from Japan (e.g. Okinaka *et al.*, 1957). It is usually encountered in young adult males and there is only rarely a family history of periodic paralysis. In the majority, the onset of the attacks coincides with or follows the onset of the hyperthyroidism and they cease with adequate treatment of the thyroid disorder. The attacks are associated with hypokalaemia, although the underlying disorder of function has been considered to differ from that of familial periodic paralysis (Engel, 1961a).

The association between *myasthenia gravis* and thyrotoxicosis is discussed later (see p. 54). Although thyrotoxicosis is encountered in about 5 per cent of patients with *myasthenia gravis*, the latter disorder is only very rarely met with in patients suffering from thyrotoxicosis.

### Muscle Disorders Associated with Hypothyroidism

Sluggish tendon reflexes with a reduction in the speed of contraction and relaxation are frequently seen in myxœdema, most easily observable in the ankle jerk. It has been demonstrated that this is not due to an abnormality of the neural components of the reflex or of muscle excitation but appears to be the result of an alteration in the contractile mechanism itself (Lambert *et al.*, 1951). A generalized increase in muscle bulk may also be evident, the muscles having a firmer consistency than normal (Hoffman's syndrome). This is associated with muscle pain and stiffness, aggravated by movement. The condition displays similarities to myotonia, but electromyographic recordings demonstrate that this is not associated with myotonic discharges and the phenomenon is therefore best termed "pseudo-myotonia". These changes are reversed by thyroxine (Wilson & Walton, 1959). A similar condition

in cretinous children has been termed the "Debré-Semelaigne syndrome".

Åström, Kugelberg & Müller (1961) have reported that a proximal myopathy may develop in patients with myxœdema, producing mild limb-girdle weakness and wasting, and myopathic electromyographic changes. The appearances in muscle biopsies were somewhat indefinite. The disorder was usually improved by treatment of the myxœdema.

### **Muscle Disorders Associated with Diseases of the Adrenal Glands**

The occurrence of a myopathy in association with Cushing's syndrome is now well recognized. The original description was made by Müller & Kugelberg (1959), although it was previously realized that muscular weakness was common in this disorder (Plotz, Knowlton & Ragan, 1952). In six patients with Cushing's syndrome, Müller & Kugelberg found weakness of the proximal leg muscles in five, two of whom also displayed weakness of the shoulder girdle musculature. Electromyographic examination demonstrated myopathic changes and muscle biopsies revealed moderate degenerative changes in the muscle fibres with some connective tissue replacement. It is of interest that the histological changes were considerably less than might have been anticipated from the clinical and electromyographic findings, as is the situation in thyrotoxic myopathy. The condition is presumably analogous to corticosteroid myopathy. The amount of improvement of the myopathic symptoms with treatment of the Cushing's syndrome is disappointing.

The episodic hypotonic paralysis that may be associated with aldosterone-secreting adrenal tumours (Conn's syndrome) has already been discussed (p. 46). Patients with Addison's disease frequently complain of weakness which is reversed by correction of the water and electrolyte disturbance. There is no evidence of a true myopathy. Contractures of the legs develop in rare instances, but these are thought to be the result of fascial and tendinous shortening and not to be of myopathic origin (Adams, Denny-Brown & Pearson, 1962). The cause of this is obscure.

### **Corticosteroid myopathy**

Perkoff and co-workers (1959) drew attention to the development of a myopathy similar to that seen in Cushing's syndrome in patients receiving long-term corticosteroid treatment. The weakness likewise involves the limb girdle muscles, particularly those of the pelvic girdle. This has since been confirmed by numerous reports. The risk is related to the duration of treatment and dosage and appears to be greatest with preparations such as triamcinolone that possess a fluorine atom in the 9 $\alpha$  position (Williams, 1959; Golding *et al.*, 1961). There are myopathic abnormalities in the electromyogram, but muscle biopsies show few changes. The serum creatine kinase level is normal (Pearce, Pennington & Walton, 1964a). In contrast to the myopathy of Cushing's syndrome, recovery is usually satisfactory if corticosteroid administration is ceased. Corticosteroid myopathy has been studied in animals (Ellis, 1956; Smith, 1964; D'Agostino and Chiga, 1966). A reversible segmental degeneration of the muscle fibres is observed.

## MYASTHENIC SYNDROMES

### Myasthenia Gravis

**Clinical Features.** These have been reviewed at length by Grob (1953, 1958) and Simpson (1958, 1960, 1964) and do not require detailed repetition. The disease is characterized by abnormal fatigueability of muscle noticed on sustained activity with improvement in power following rest. In most cases, the condition begins between the ages of 15 and 35. When cases arise in the younger age groups, females are affected considerably more frequently than males (4.5 : 1), but in the late onset cases, males predominate (2 : 1). The overall incidence in women is twice that in men. The disease begins either gradually or fairly abruptly. The external ocular muscles are involved more often than any other muscle group, the nuchal, limb girdle, facial, masticatory and bulbar muscles being affected next in order of frequency.

The course of the disorder has been the subject of a number of studies in recent years (Grob, 1953, 1958; Ferguson, Hutchinson & Liversedge, 1955; Simpson, 1958, 1960). Marked fluctuations in severity may occur initially but are infrequent if the disease has been present for 5–7 years. Sudden deterioration may take place (“myasthenic crisis”) and may imperil life because of weakness of the respiratory muscles. Complete remission may be observed, sometimes lasting several years. If a substantial remission is to occur, it is usually seen within the first year. More than one complete remission is rare. Myasthenia localized to the external ocular muscles may be encountered, especially in males, and if it has not extended within two years, it has been suggested that it is then unlikely to do so. In long-standing cases, persisting proximal muscle weakness and wasting in the limbs may supervene (myasthenic myopathy) and weakness of the external ocular muscles that does not respond to anticholinesterase preparations.

**Association with other Disorders.** Myasthenia gravis is associated with thymic carcinoma in about 10–20 per cent of cases. The age of onset in such cases tends to be later and the predominance of female over male cases is not so marked (Keynes, 1955; Perlo, Schwab & Castleman, 1965). Hyperthyroidism coexists in about 5 per cent of cases (Millikan & Haines, 1953; Silver & Osserman, 1957) and the prognosis in such cases appears to be worse. The time of onset of the two conditions is not closely related. An inverse relationship between the severity of the myasthenic and hyperthyroid symptoms has been claimed, but seems unlikely.

Although not accepted by all authorities, treatment of the hyperthyroidism has in general led to improvement of the myasthenia. Engel (1961b) found that myasthenia gravis is aggravated by hypermetabolism induced by the administration of either thyroid hormones or TSH and that the effect therefore appears to be the result of thyroid hormone itself. Hypothyroidism may also exist in association with myasthenia gravis.

A number of other conditions may be encountered in patients with myasthenia gravis, although a more than chance association has not yet been fully established. In this respect, rheumatoid arthritis and systemic lupus erythematosus have been emphasized as of particular interest (Simpson, 1960).

**Neonatal Myasthenia.** Transient myasthenia is occasionally observed in

children born to myasthenic mothers (see Stern, Hall & Robinson, 1964). Generalized weakness and reduced spontaneous movement are noticeable within a few hours of birth and persist for up to three weeks. It may be severe enough to cause death, but responds satisfactorily to neostigmine (Millichap & Dodge, 1960).

**Pathology and Pathophysiology.** Pathological changes in muscle in myasthenia gravis are often inconspicuous. Those that occur have been analysed by Russell (1953). Three types of change were observed: an acute necrosis of muscle fibres with accompanying inflammatory reaction, leading to loss of fibres; progressive atrophy of individual fibres with lymphorrhage formation; and simple atrophy of individual fibres. These abnormalities were not considered to be peculiar to myasthenia and were seen in cases with and without thymoma. Changes in the nerve terminals and motor end-plates have been described (Coërs & Woolf, 1959; Bickerstaff & Woolf, 1960) and are also seen in clinically unaffected muscles (MacDermot, 1960). Two kinds of abnormality are found. In one, which is not considered specific for myasthenia, the terminal knobs of the axons are shrunken and there is increased sprouting from the subterminal axon. In the other, which may be specific to myasthenia, the terminal knobs are few in number, may be arranged serially along the terminal branches of the axon and are related to unusually elongated end-plates. Electron microscope observations have shown no obvious alteration in the number of synaptic vesicles (Bickerstaff, Evans & Woolf, 1960); this is of interest as they may contain the transmitter substance. The thymus is abnormal in a high proportion of cases, even when no thymoma is present, and contains numerous "germinal centres" (Castleman & Norris, 1949).

The nature of the disturbance of neuromuscular transmission is still uncertain. The fact that improvement results from the administration of anticholinesterases eliminates the possibility of a depolarization block at the end-plate, but would be compatible with a failure of acetyl choline synthesis or release, excessive hydrolysis of acetyl choline once released, a competitive block at the end-plate or an abnormality of the end-plate itself. Estimations of cholinesterase activity, both in the blood and at the end-plate have failed to demonstrate any excess and this possibility can therefore be eliminated. The possibility of a pre-synaptic defect was raised by Desmedt (1958), who argued that the electrical features of the neuromuscular block resemble those produced by hemicholinium, which interferes with the synthesis of acetyl choline. Similarly, studies on the spontaneous miniature end-plate potentials in biopsies also led Dahlbäck and his co-workers (1961) to believe that the defect was pre-junctional. On the other hand, abnormalities in the response of the end-plates to decamethonium (Churchill-Davidson & Richardson, 1952) and acetyl choline (Grob, Johns & Harvey, 1955) have been demonstrated, but it is not yet certain whether they represent a primary disturbance or whether they are secondary to long-standing acetyl choline deficiency.

Although claims have been made for the presence of a circulating blocking agent, none has so far been adequately demonstrated, and the same is true of thymic extracts. A circulating factor of some kind that is able to cross the placental barrier is the most plausible explanation of neonatal myasthenia. Simpson (1960) has suggested that this is an antibody to the end-plate

receptors having the property of a competitive blocking agent for acetyl choline. Myasthenia gravis could then be considered as an example of "auto-immune" disease. This would explain its occasional association with other disorders thought to have a basis in auto-immunity such as Hashimoto's thyroiditis and systemic lupus erythematosus. The weight of the evidence at present available, however, favours a pre-junctional defect.

**Diagnosis.** Confirmation of the diagnosis is best made by the intravenous injection of the short-acting anticholinesterase preparation edrophonium chloride (Osserman & Kaplan, 1952). Improvement in muscle power, if this occurs, is observed within a minute. If a definite result is not obtained, an intramuscular injection of neostigmine may be employed, testing muscle power before and 15–30 minutes after injection.

Electromyographic testing may also be performed. The electromyographic abnormalities in myasthenia gravis have been reviewed by Simpson (1966). The responses to decamethonium (Churchill-Davidson & Richardson, 1952) and curare have been suggested as diagnostic measures, but have not been widely employed.

**Treatment.** The two most widely used anticholinesterase preparations are neostigmine bromide and pyridostigmine bromide. The duration of action of the latter is more prolonged, although the difference is not as great as was originally supposed. The longer action of pyridostigmine is useful in supplying a more sustained effect, and the shorter acting neostigmine may be given before any additional activity undertaken by the patient. Ambenonium chloride has been introduced in recent years and has a slightly more prolonged action than pyridostigmine, making the possibility of cumulative effects somewhat more likely. The use of spironolactone was recommended by Gottlieb & Laurent (1961). They suggested that its action in causing potassium retention would increase end-plate sensitivity to acetyl choline, but it appears to be of uncertain usefulness. This comment also applies to ephedrine, which has been employed for many years as an adjuvant to neostigmine.

Patients receiving anticholinesterase preparations may experience abdominal pain and diarrhoea because of muscarinic side effects, but these are not normally troublesome. Excessive dosage can lead to the development of a depolarization block at the end-plates and this may be difficult to differentiate from a worsening of the myasthenic state. The injection of edrophonium may be helpful in distinguishing between the two (Osserman & Kaplan, 1953), as improvement may be detectable if the weakness is myasthenic and worsening if it is the result of a cholinergic blockade. However, unequivocal results are not always obtained and the procedure is not without danger if the respiratory muscles are involved. If doubt exists, it is best to withdraw medications completely under circumstances where assisted respiration can be given if necessary and then to reinstitute treatment gradually later when an improvement on edrophonium administration can be demonstrated.

The situation with respect to treatment by thymectomy is still somewhat unsatisfactory as no controlled trial has ever been undertaken. In patients with myasthenia gravis associated with a thymoma, removal of the thymoma, although at times followed by a temporary alleviation of the myasthenia, does

not usually produce a lasting benefit. In patients without a thymoma, Simpson (1958), from observations on a large series of cases, concluded that the operation may be of benefit in younger women with a history of myasthenia of less than seven years in duration. No significant difference was demonstrated in men. A similar conclusion was recently reached by Henson, Stern & Thompson (1965) and Perlo *et al.* (1966).

#### **Myasthenic Syndrome Associated with Bronchial Carcinoma (Eaton-Lambert Syndrome)**

The occurrence of a myasthenic syndrome differing from myasthenia gravis in patients with small-celled carcinoma of the bronchus has been recognized in recent years (Anderson, Churchill-Davidson & Richardson, 1953). Its features were defined by Eaton & Lambert (1957) and Wise & MacDermot (1962), and have recently been reviewed by Lambert & Rooke (1965). It is characterized by weakness and fatigueability of the proximal limb muscles, particularly those of the pelvic girdle and thighs. The external ocular, facial and bulbar muscles are sometimes also involved. A transient improvement in muscle power after activity may be noticed. The tendon reflexes are depressed or unobtainable. The response to neostigmine is usually poor, particularly in the later stages and there is a greatly heightened sensitivity to curare.

The electrical features of the neuromuscular block are of diagnostic importance. The degree of block is greater in the rested muscle than after activity, so that the muscle action potential evoked by a single maximal electrical stimulus to the motor nerve is considerably greater after a short period of vigorous contraction or repetitive electrical stimulation to the motor nerve. Although such post-tetanic potentiation is seen in myasthenia gravis, its magnitude is much greater in the myasthenic syndrome associated with bronchial carcinoma. The cause of the failure in neuromuscular transmission is not yet known, but the features resemble those produced by botulinum toxin, which is known to interfere with the release of acetyl choline from the nerve terminals.

#### **Abnormalities of Neuromuscular Transmission in other Conditions**

Muscle fatigueability that is improved to some extent by the administration of anticholinesterase preparations has been reported in disorders involving the lower motor neuron and in polymyositis. This has been investigated electromyographically and a mild defect of neuromuscular transmission has been demonstrated in dermatomyositis, polymyositis, systemic lupus erythematosus, poliomyelitis, motor neuron disease and some examples of peripheral neuropathy (see Simpson, 1966).

It is of interest that neuromuscular transmission in the first few weeks of life differs from that found in the normal adult, showing some of the features of myasthenia (Churchill-Davidson & Wise, 1963).

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# CHAPTER 3

## COAGULATION DISORDERS

by

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BLEEDING from a cut vessel is stopped by three closely related mechanisms—a vascular reaction, a platelet response and coagulation. Disorders of coagulation can be due to an inborn deficiency of a coagulation factor or can be secondary to a variety of acquired diseases.

### Nomenclature

Present concepts of physiological clotting have largely evolved from studying patients with inborn defects. From these studies ten genetically determined clotting factors have been identified. The first step in the identification of a clotting factor has usually been the preparation from normal blood of a fraction which corrects the clotting defect in the abnormal sample.

TABLE 3.I

INTERNATIONAL NOMENCLATURE OF CLOTTING FACTORS WITH OLD NAMES  
AND RELATED DEFICIENCY STATES

Factor number	Names	Deficiencies	
		<i>Congenital</i>	<i>Acquired</i>
I	Fibrinogen	Hypo- and afibrinogen- æmia	Liver disease Acute bleeding states Extensive bone marrow disease
II	Prothrombin	Hypoprothrombinæmia	Liver disease Vit. K deficiency
III	Tissue thromboplastin	—	—
IV	Ionized calcium	—	—
V	Labile factor Proaccelerin	V deficiency	Liver disease Acute bleeding states
(VI	Accelerin. <i>Not now used</i> )	—	—
VII	Proconvertin	VII deficiency	Vit. K deficiency
VIII	Anti-hæmophilic factor	Hæmophilia von Willebrand's disease	Acute bleeding states
IX	Christmas factor	Christmas disease	Liver disease Vit. K deficiency
X	Stuart factor	X deficiency	Liver disease Vit. K deficiency
XI	Prothromboplastin antecedent	P.T.A. deficiency	—
XII	Hageman factor	XII deficiency	—
XIII	Fibrin stabilizing factor	XIII deficiency	—

This may involve elaborate physical and chemical processing—inevitably more elaborate as each new factor is discovered. In clarifying the growing terminological confusion, the work of the International Committee on Blood-clotting Factors is a major advance (Wright, Koller & Streuli, 1960; Wright, 1962). The Committee recommended the use of Roman numerals to distinguish distinct and individual factors; and, although such names as fibrinogen, prothrombin and thrombin are still used, their recommendations have been widely adopted. Conditions such as hæmophilia and Christmas disease, for example, are now described as factor VIII and factor IX deficiency. Table 3.I gives the old and new nomenclatures and the clinical syndromes associated with the various deficiency states.

The new numbering does not reflect the order of events in physiological coagulation. The recently added factor XIII, for example, contributes to the stability of the fibrin clot and must therefore follow on the activation of factor I (fibrinogen). Factor VI, on the other hand, has been eliminated since it has been shown to be indistinguishable from the active form of factor V. The place and identity of an apparently new plasma thromboplastin factor reported by Hathaway, Belhasen & Hathaway (1965), of hæmophilia B<sub>M</sub> (Houghie & Twomey, 1967) and of a number of newly described fibrin fractions remain to be established.

### THE MECHANISM OF COAGULATION

Blood can clot through two different pathways—one *intrinsic* and the other *extrinsic* (Fig. 3.1). The intrinsic system, which takes place within the blood vessel, is dependent on the constituents of the blood itself. This system, initiated by contact of the blood with any surface which differs from normal

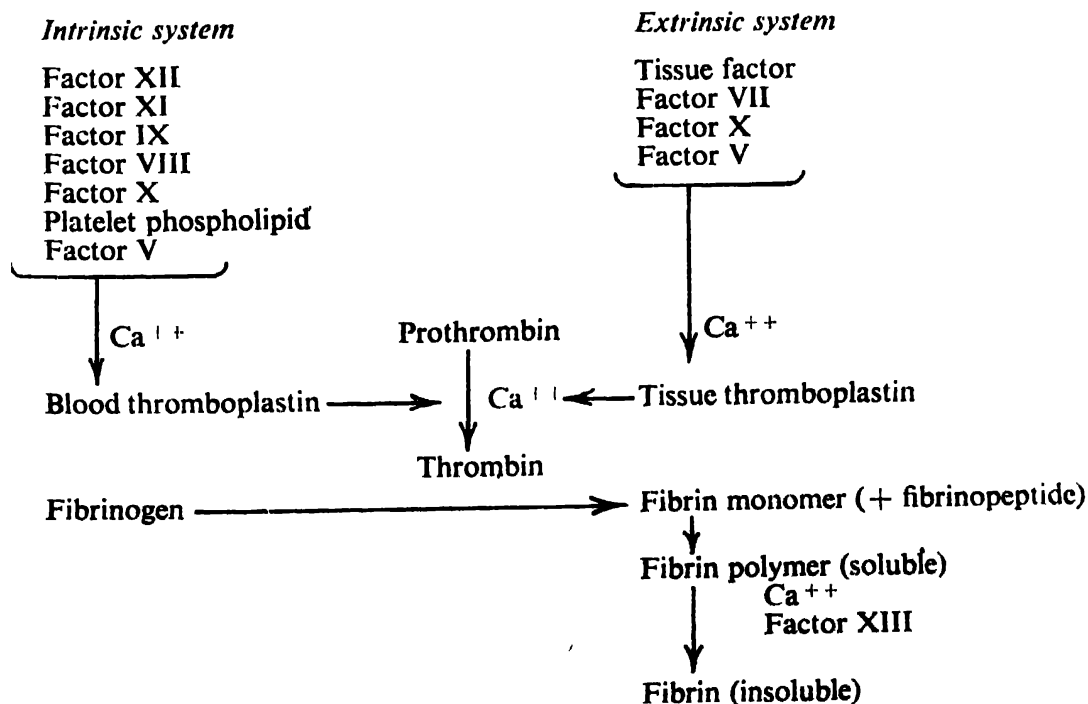
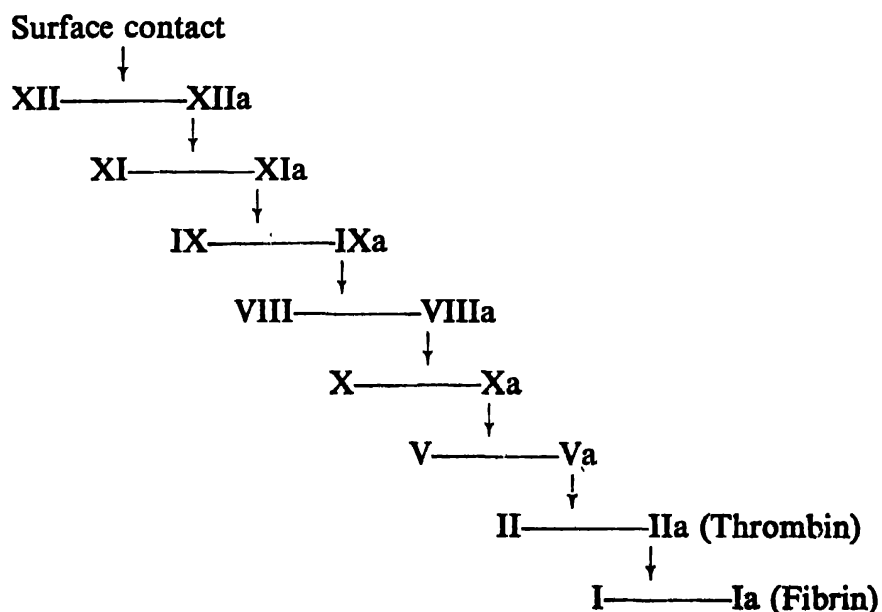


FIG. 3.1. The blood coagulation mechanism. (Reproduced from "Bleeding Disorders. Investigation and Management". By R. M. Hardisty & G. I. C. Ingram (1965), by courtesy of Blackwell Scientific Publications, Oxford.)

vascular endothelium, leads (following a chain of reactions among inactive precursors) to the formation of blood thromboplastin and hence to a fibrin clot. In the laboratory, the most common activating surface is glass but kaolin, silica and other particulate matter are all used to achieve standard degrees of activation. If contact with a foreign surface is prevented by the use of siliconed material, this will not take place. The extrinsic system depends on the presence of tissue factor as well as of blood. It leads to the formation of tissue thromboplastin and hence to clot formation *outside* the blood vessels. The two mechanisms can be investigated more or less independently. Such procedures as the partial thromboplastin time and the thromboplastin generation tests reflect the efficiency of the intrinsic mechanism, provided that the prothrombin time is normal, whereas the one-stage prothrombin time and its variants measure the factors which play a part in the extrinsic process.

Macfarlane has suggested that the succession of events can be conceived in terms of the sequential activation of an "enzyme cascade" (Macfarlane, 1964, 1965). According to this hypothesis each stage represents an enzyme-catalysed reaction in which the product of one reaction becomes the enzyme of the next.

With the suffix (a) denoting the activated (enzyme) form, the sequence from surface contact to fibrin formation is set out as follows:



At each successive stage an increasing amount of pro-enzyme is involved; suggesting that the whole sequence of pro-enzyme/enzyme transformation functions as a biochemical amplifier in which the minute initial stimulus of physical contact is transformed into the outburst of thrombin activity required for efficient hæmostasis. The slower generation of thrombin, which occurs in hæmophilia and similar conditions and which is the cause of abnormal bleeding, is the result of the loss of power in one of the early stages of this system of amplification (Macfarlane, 1966). A comprehensive scheme in which all stages are represented is shown in Fig. 3.2.

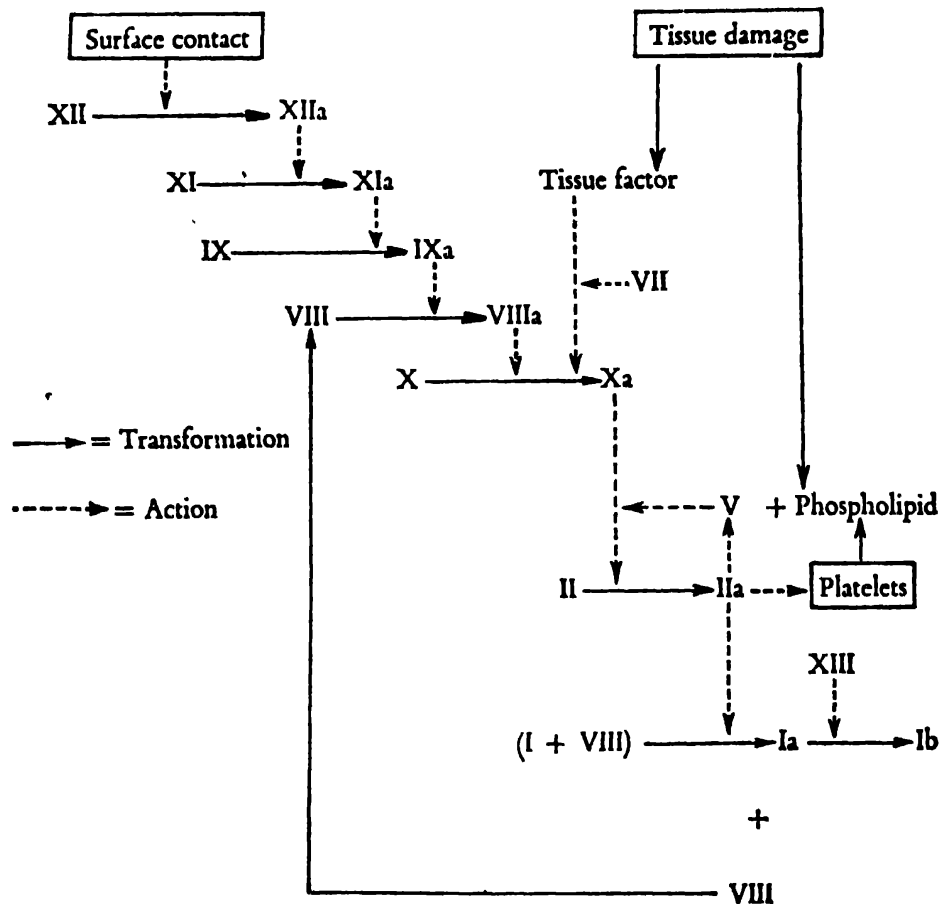


FIG. 3.2. A blood coagulation scheme based on existing evidence, and including the autocatalytic effects of thrombin. Ia = fibrin and Ib = stabilized fibrin. (Reproduced from "Treatment of Hæmophilia and other Coagulation Disorders", R. Biggs & R. G. Macfarlane, 1966, by courtesy of Blackwell Scientific Publications, Oxford.)

### THE HÆMOSTATIC BALANCE AND PHYSIOLOGICAL FIBRINOLYSIS

Several well defined mechanisms are available to preserve the fluidity of the blood. These include the integrity of normal vascular endothelium, normal blood flow, the presence of physiological anti-coagulants in the blood (Soulier, 1961), the destruction of activation products by the liver (Spaet & Cintron, 1960; Spaet, 1962) and physiological fibrinolysis (Fearnley & Tweed, 1953).

Fibrinolysis, like coagulation, is the end result of a multistage reaction. It is the natural way in which formed fibrin is destroyed and blood clots are dissolved. Under physiological conditions fibrin formation and fibrinolysis are in a state of dynamic balance.

The fibrinolytic system has four main components: plasminogen, the inactive precursor substance which circulates in plasma; plasminogen activators; plasmin, the active fibrinolytic enzyme; and plasmin inhibitors (anti-plasmins).

Activators and inhibitors of fibrinolysis can be differentiated and



separated in various ways. Plasminogen and plasmin, for example, are present in the globulin fraction of blood, whereas anti-plasmins are in the albumin fraction (Macfarlane & Pilling, 1946; Flute, 1960; Green & Thomson, 1962).

Activators are present in the plasma of normal subjects; the amount is very variable and is increased by exercise and stress. They have also been isolated from most tissues, particularly the uterine endometrium, the lungs, the prostate, the thyroid and from the urine. Urokinase, the activator present in urine, has been investigated by Astrup & Steindor (1952). Pharmacological fibrinolysis can be instigated by a number of substances. In the treatment of occlusive vascular disease streptokinase, isolated from the filtrates of hæmolytic streptococci, was the first plasminogen activator to become available. Clinical indications for therapy (Douglas & McNicol, 1964) include pulmonary embolus, occlusion of major limb arteries and thrombosis of the retinal vessels. Urokinase and streptokinase are preformed activators which, if given in large enough doses, will dissolve thrombi, although vital tissues are often dead before the clot is penetrated. Increase in the naturally occurring plasma activators can be produced by drugs like Phenformin and Metformin. These do not achieve a concentration sufficient to dissolve major preformed thrombi. Their use is more likely to have a place in prophylaxis.

Naturally occurring inhibitors of fibrinolysis in the form of antiplasmins which act on the plasmin direct are well recognized (Norman, 1960). The existence of physiological inhibitors of plasminogen activation is not proven. Fibrinolytic inhibitors for therapeutic use are discussed with management of pathological fibrinolysis. The whole subject was fully reviewed in the *British Medical Bulletin*, Vol. 20, No. 3, 1964, and by Fearnley (1964, 1965).

### HÆMOPHILIA

With the exception of factor XII, all inborn clotting-factor deficiencies tend to result in a more or less severe bleeding syndrome. Hæmophilia (factor VIII deficiency) is considered in greater detail than the other coagulation disorders because it is relatively common (the number of patients in this country is estimated at between two and three thousand), and because recent advances in treatment have markedly improved the outlook even of those most severely affected.

#### Heredity

It has long been recognized that hæmophilia is transmitted by a sex-linked recessive gene. As a general rule not only the clinical severity but also the factor VIII levels of affected males within any one family tend to be remarkably constant.

Much work has been directed in recent years toward the detection of carriers. On genetic grounds alone a carrier can be identified with certainty (a) if she is the daughter of a hæmophiliac, (b) if she is the mother of one hæmophilic son and appropriately related to another hæmophiliac or proven carrier, (c) if she is the mother of more than one hæmophilic son. (A single affected son might be the result of a mutation.) If a girl's mother is a carrier she has an even chance of being a carrier herself. If only her grandmother is

known to be a carrier, her chances are one in four. Laboratory diagnosis of the carrier state in a woman appropriately related to a hæmophiliac can be attempted only by assaying the plasma factor VIII level. The answer is rarely clear-cut. If the assay, carried out under rigorously controlled conditions, shows a factor VIII level of less than 30 per cent—the woman is probably a carrier. A normal factor VIII level, however, does not exclude the carrier state.

The distribution curves for factor VIII levels among genetically proven carriers and normal females have been studied by Kerr and co-workers (1965), who have interpreted their finding (Fig. 3.3) in terms of the Lyon hypothesis of X-chromosome function. One assumption of this hypothesis, with which their results agree, is that a group of females who are heterozygous for the

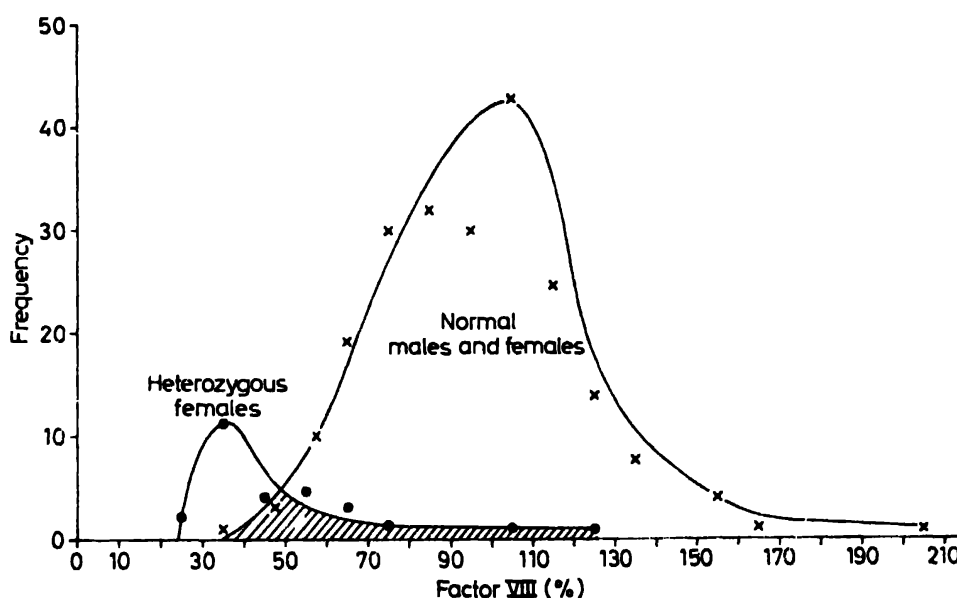


FIG. 3.3. Approximate distribution curves for factor VIII levels in genetically proven heterozygous females.  
(Reproduced from *Genetics and the Interaction of Blood Clotting Factors* (1965), "The inheritance of Factor VIII", by C. B. Kerr and co-workers, by courtesy of the publishers, Friedrich-Karl Schattauer Verlag, Stuttgart.)

abnormal X-linked gene should show a mean level half-way between the mean in a group of affected males and the mean in a group of normal females. On this basis it would be expected, as is seen to be the case, that a proportion of genetic carriers have factor VIII levels which overlap with the lower range of those for normal females.

While at low factor VIII levels the assay results are remarkably reproducible, at higher levels extraneous influences cannot be excluded. Some of these variations probably reflect genuine fluctuations in factor VIII synthesis, e.g. the changes associated with exercise or pregnancy. Others are mainly technical. The main source of error in the second category is the difficulty of standardizing control plasmas. The data of Kerr and co-workers (1965, 1966) suggest that variations in factor VIII levels in normal families depend partly on non-genetic influences and partly on the influence of an unknown number of autosomal genes.

### Diagnosis

As with most inborn bleeding disorders a tentative diagnosis can often be made on the history. Firm diagnosis can only be based on the finding of a low plasma factor VIII level. There is a remarkably close correlation between this level and the clinical severity of the illness. At one extreme, severe hæmophiliacs who have a factor VIII level of less than 1 per cent tend to bleed during the course of everyday life without being subjected to any particular trauma. In such cases some degree of crippling was inevitable in the past and even with modern therapy the disease can still become incapacitating. At the other extreme, mild hæmophiliacs with factor VIII levels of 5–30 per cent bleed only after accidental or surgical trauma. In such patients the better outlook may, to some extent, be counterbalanced by the greater risk of dying from a single catastrophic hæmorrhage, for it is often insufficiently recognized that, without adequate therapy, a “mild bleeder” is as likely to bleed to death from an operation as the most severely affected person.

Factor VIII assays are outside the repertoire of many hæmatological laboratories, but a presumptive diagnosis can be based on comparatively simple screening tests. In disorders of the intrinsic clotting mechanism the prothrombin time is normal and the partial thromboplastin time is abnormal. If plasma from a known hæmophiliac is available the diagnosis can be strengthened by showing that the partial thromboplastin time is corrected by the addition of normal but not by the addition of hæmophilic plasma (Matchett & Ingram, 1964). Classically, and rather more laboriously, the diagnosis can be made by the thromboplastin generation test which shows an abnormal plasma but normal serum components. Screening tests can also be performed on capillary samples, using diluted blood (Dormandy & Hardisty, 1961).

Although these tests can prove extremely useful in initial investigation, or in an emergency, all patients should be referred to one of the designated Hæmophilia Centres so that complete investigations can be carried out, an official Hæmophilia Card be issued, and arrangements be made for future therapeutic cover.

### Sites and Types of Bleeding

In both hæmophilia and Christmas disease the commonest bleeding sites are the joints and the soft tissues. Joint bleeds may appear minor, but, if inadequately treated, they inevitably lead to severe crippling and painful arthropathy. Soft-tissue bleeds, on the other hand, often present as acute emergencies: in the neck they can cause compression of the air passages, and in the forearms and legs they can rapidly lead to irreversible Volkmann-type contractures. Retroperitoneal hæmorrhage is the commonest cause of an “acute abdomen” in hæmophiliacs—a fatal complication unless treated promptly or if it leads to misguided surgery. When present, an area of anæsthesia referable to the cutaneous branches of the femoral nerve is a useful differentiating sign which suggests bleeding into the psoas muscle (Biggs & Matthews, 1966a). The actual site of hæmorrhage giving rise to lesions in the groin (i.e. whether it is in the joint or the surrounding muscle—e.g. ileo-

psoas) can seldom be identified with certainty. Immediate treatment is indicated, for amongst the other hazards, blood, tracking down the muscle sheaths, is apt to form blood cysts and pseudo-tumours later necessitating an amputation of the limb (Abell & Bailey, 1960; Valderrama & Matthews, 1965).

Headache in hæmophilia presents a difficult diagnostic problem. Hæmorrhage is a likely cause, and Fessey & Meynell (1966) recommend giving prophylactic factor VIII replacement if a headache has persisted for more than 12 hours. By the sustained and adequate administration of anti-hæmophilic factor concentrates Davies and co-workers (1966) have safely used standard techniques (including carotid angiography on seven occasions) for the investigation and management of five hæmophiliacs with intracranial lesions.

Repeated gastro-intestinal hæmorrhage calls for a difficult therapeutic decision. As in non-hæmophiliacs, the commonest cause is peptic ulceration; the risks of an operation must be weighed against those of ill-controlled bleeding. In the series of Carron, Boon & Walker (1965), 12 out of 14 hæmophiliacs with gastro-intestinal hæmorrhage had peptic ulcers which could be demonstrated either radiologically, at operation, or at necropsy, and in half the cases hæmorrhage was the only symptom in the early stages. After recurrent bleeds nearly half the patients had to have emergency surgery. It seems fair to conclude that the development of a duodenal ulcer in a hæmophiliac is a strong indication for elective surgery in a unit adequately equipped to deal with the bleeding tendency. The diagnosis may reasonably be based on radiological evidence or on the clinical findings alone (Biggs & Matthews, 1966b).

Deep cuts or surgical trauma (including dental extractions) may be followed by persistent oozing for weeks. The onset is sometimes delayed for several days and the bleeding is often intermittent. A deceptive feature of this type of hæmorrhage is that large friable clots often form in the wound or tooth socket. These are the products of the extrinsic coagulation mechanism (which is unimpaired): they are extravascular and are not only ineffective as a hæmostatic plug, but may actually retard healing.

### Management

The management of hæmophilia, is simple in principle and exacting in practice. *Replacement of the missing clotting factor* is necessary to enable the blood to clot; temporary *immobilization* of the part is needed to prevent further hæmorrhage as the level of the clotting factor falls. Immobilization calls for a good deal of care and common sense. Bleeding parts should always be rested, but never in such a way as to interfere with their observation. The extent of immobilization ranges from strict bed rest, which must be enforced after a retroperitoneal hæmorrhage or abdominal operation, to local splinting for recurrent hæmarthroses, and dental splinting after extractions. Improved splinting has been achieved with the use of new plastic materials.

### Plasma Replacement Therapy

The regime of frequent large plasma transfusions, as outlined by Brinkhous and co-workers (1956), based on the assumption that the *in*

*vivo* half-life of factor VIII is 6–8 hours, has been modified by technical advances. The object of such infusions, now often referred to as plasma replacement therapy, is to raise the patient's circulating factor VIII above a critical low level as quickly as possible.

The principle of giving an infusion of plasma or concentrate at the onset of trouble should be applied to almost every situation which might be due to hæmorrhage. It cannot be too strongly emphasized that the best and sometimes the only judge of the onset of internal bleeding is the patient himself. Where doubt exists as to the diagnosis—in the case of an *acute abdomen* or *meningeal irritation*, for example—it is often advisable to give suitable antibiotics as well as the infusion, even though the double therapeutic approach may mask the diagnosis. Under no circumstances should the abdomen be palpated until adequate plasma replacement has been given; even then, this must be done extremely gently. If antibiotics are initially withheld, the patient's response to the first infusion will be of diagnostic value as well as therapeutic. If the underlying lesion is a hæmorrhage, the symptoms lessen when correction of the clotting defect controls the hæmorrhage. If, in the case of an acute abdomen, the symptoms are due to appendicitis, they are unlikely to regress with plasma replacement therapy alone. In every case the dose response should be tested after the first infusion for this information will be vital if operation is considered.

*Joint and muscle hæmorrhages* need not be painful at their onset. The patient's description of a stiffening joint or of something "giving" may be highly significant. If plasma replacement therapy is delayed until obvious signs appear, there is a grave risk that permanent arthropathy will occur; this may necessitate long and painful periods in hospital for remedial therapy. The immediate benefits of the early treatment of musculo-skeletal hæmorrhage include relief of pain, shortening of the time taken for resolution of the hæmorrhage and, consequently, a marked reduction in the time away from school or work. Figure 3.4 shows an estimate of "time out of action" given by patients for hæmarthroses treated with, and without, plasma replacement (Ali *et al.*, 1967). A further complication is that one bleed leads to another and it is this succession which will immobilize him for many years. Adequate treatment of the first episode not only forestalls the entire chain of events (Katz & Husek, 1965; Katz, 1966) but leads to a sharp drop in the incidence of cases requiring orthopædic rehabilitation (Jordan, 1965). A full appreciation of the long-term benefits of intensive plasma replacement therapy in the prevention of arthropathy will be more apparent when this newly treated generation of hæmophilic boys has grown up. Strong support for the view that plasma therapy does prevent serious arthropathy is provided by the work of Brinkhous (1966) and by reports on the Swedish boys who are being given Blombäck fraction I-O, at 2–3 week intervals, as prophylactic therapy (Ahlberg, 1965; Blombäck, 1967). As is to be expected, the best results are seen in patients in whom treatment was started before permanent arthropathy occurred; therapy must, therefore, be available from early childhood.

The indication for hospital admission and immobilization depends not only on the severity of the lesion but also on whether it is a recent recurrence. In particularly severe or recurrent joint hæmorrhage, replacement therapy during mobilization will often allow earlier and safer weight-bearing. During

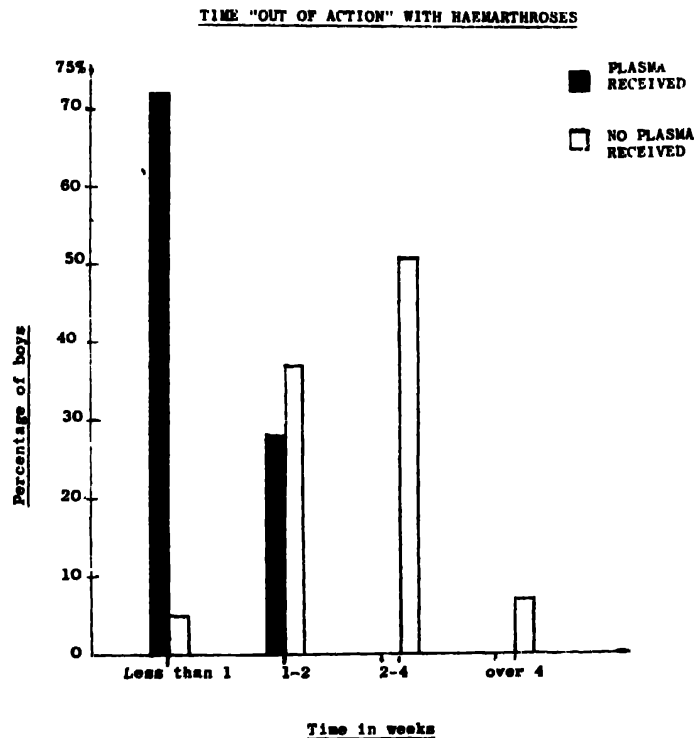


FIG. 3.4. The effect of plasma replacement therapy in reducing the "time out of action" with hæmarthroses.

rehabilitation, carefully graded physiotherapy is as important as early immobilization.

*Hæmaturia*, though often frightening to the patient, frequently stops spontaneously; it need seldom be treated with the same degree of urgency as other types of hæmorrhage. Oral iron should be given to prevent iron deficiency, and in severe anæmia a transfusion of packed cells may be indicated. Biggs & Matthews (1966) have outlined a regime for the treatment of persistent hæmaturia in which plasma, in doses of 16–20 ml per kg wt., is given for three consecutive days. If bleeding continues after this, concentrated human factor VIII should be given on the fourth and possibly fifth days. Where AHF-rich cryoprecipitate is available, this concentrate can, of course, be given with advantage on the first day.

*Speed in infusing* the plasma or plasma concentrate is essential. One litre of plasma, for example, must be run into the vein within 30–60 minutes (concentrate, of course, is given even more quickly). Even when an anæmic patient is in need of blood as well as factor VIII, the fresh plasma should be separated and the appropriate volume given rapidly first and the packed cells more slowly later. The average *single dose* of fresh plasma needed for the treatment of a soft tissue bleed is 15–20 ml per kg body-weight.

None of the preparations so far available can be relied on absolutely to provide a potent source of the clotting factor; Bennett and co-workers (1967) have drawn attention to the poor quality of "fresh-frozen" plasma issued throughout wide areas of S.E. England for the routine treatment of hæmophilic hæmorrhage. Ideally, therefore, all infusions should be carried out under laboratory control—i.e. both the preparation administered and the patient's

dose response should be tested by factor VIII assays. Provided that these tests are never omitted in the case of surgical procedure or serious hæmorrhage (cerebrovascular, abdominal, etc.), that the therapeutic material supplied is tested regularly and that each patient's dose response is tested intermittently, it is reasonable to assess the efficacy of treatment by the symptomatic response. In the case of joint, muscle and soft tissue hæmorrhage there is fortunately close correlation between the efficacy of treatment as manifest by a rise in circulating factor VIII and the relief of pain. If pain from this type of bleed is not relieved within two hours of starting the infusion, or if hæmorrhage of any kind has obviously not come under control, the potency of the preparation must be questioned or the development of an inhibitor suspected.

### Sources of Factor VIII

(a) *Fresh and Fresh-frozen Plasma.* Amongst possible sources of factor VIII, fresh citrated plasma and properly made fresh-frozen plasma are satisfactory for the treatment of lesions such as musculo-skeletal hæmorrhage, in which the levels of factor VIII to be attained need not rise above 15–25 per cent. Fresh-frozen plasma is defined both by the American Association of Blood Bank Standards (1966) and by Preston (1967) as plasma processed within four hours of blood donation. Unless the level of factor VIII of the plasma used is above 60 per cent, the plasma will not be therapeutically effective; the time taken in its preparation is a vital factor influencing this (Wolf, 1959; Preston, 1967).

(b) *Human Concentrates of Factor VIII.* Since the patient's circulatory capacity limits the volume that can be infused, concentrates are needed both for the control of the more serious lesions and as prophylaxis to cover surgical procedures.

Until recently, the only human factor VIII concentrate available in the United Kingdom was the freeze-dried Human Anti-hæmophilic Factor prepared by the method of Kekwick & Wolf (1957). This is available in strictly limited quantities from the Blood Products Laboratory of the Lister Institute, England, and from the South-East Scotland Regional Transfusion Centre. For local distribution, it is also prepared on a small scale at certain regional Blood Transfusion Centres and at one or two Coagulation Centres. In 1965 Pool described the preparation and use of a concentrate of anti-hæmophilic globulin by cryoprecipitation using a closed plastic blood bag system (Pool & Shannon, 1965). This method is based on the observation that when rapidly frozen plasma is thawed in the cold the anti-hæmophilic factor precipitates with the cryoglobulin fraction. By further manipulations, the precipitate can be separated from the supernatant plasma which is either returned to the red cells and used by the routine blood bank, or used for blood volume replacement or for the correction of various other clotting factors, e.g. factor IX (Simson, 1967) or factor XI (Bennett & Dormandy, 1966). The main factors limiting the availability of this form of concentrate are the staff and organization necessary to produce it. The preparation of Pool cryoprecipitate does not involve the use of costly apparatus apart from the need for a refrigerated centrifuge.

Early reports on the clinical use of cryoprecipitate are numerous (Djerassi

*et al.*, 1965; Simson *et al.*, 1966, 1967; Hattersley, 1966; Barrett *et al.*, 1967; Bennett *et al.*, 1967; Brown *et al.*, 1967; O'Brien, 1967; Prentice *et al.*, 1967). Cryoprecipitate cannot be given intramuscularly (Pool *et al.*, 1966).

As yet, it is not possible to assess the potency of cryoprecipitate before use. Different methods of production affect potency. The choice of blood donated more than four hours before processing, or the use of glass blood-bottles, will inevitably reduce this and, therefore, affect the dosage scheme. Pool's rule of thumb dosage which aims to raise and maintain the factor VIII level above 30 per cent (Hattersley, 1966) recommends an initial priming dose of one unit or bag of cryoprecipitate—i.e. the material from one pint of blood—for each 6 kg body-weight, followed by half that amount 12-hourly for the duration of treatment. Simson (1966) and Bennett and co-workers (1967) worked out a satisfactory dosage scheme in which one bag of cryoprecipitate is taken to be equivalent to 150 ml fresh plasma, while Brown and co-workers recommend the following formula:

Number of packs required

$$= \frac{\text{Factor VIII rise intended (\%)} \times \text{body-wt. (kg)} \times 4}{1,000}$$

The development of the Pool technique represents a major advance in the management of hæmophilia. Because there need be no waste of blood when the plastic blood-bag system is used the possibility now exists of having virtually unlimited supplies of concentrated factor VIII. Pool's method is not, of course, the only one in which whole blood can be used economically (Cumming *et al.*, 1965, 1967; Johnson *et al.*, 1967), but it would certainly provide the easiest method, especially if the plastic blood-bag system were to be adopted on a national scale.

(c) *Animal Anti-hæmophilic Globulin*. As an alternative to human concentrate, animal AHG (of bovine or porcine origin) is available commercially. The preparation and clinical trials of animal AHG were initially carried out at Oxford (Macfarlane, Biggs & Bidwell, 1954). It is more potent than the human AHF and is available in unlimited quantities, but it is strongly antigenic. As a rule, each animal concentrate can be used only for 9–14-day periods; but contrary to initial fears, recent evidence suggests that the specific antibodies to animal AHG do not necessarily persist (Biggs, 1966). Thrombocytopenia is an occasional complication, more common after bovine than after porcine AHG. Animal AHG should be reserved for the control of major surgical procedures. Details for its use are given by Biggs (1966).

### Major Surgery

Planned major surgery should be carried out only at the major Hæmophilia Centres. Management of the surgical case is based on raising the patient's factor VIII level to between 60–100 per cent, before the operation, and on maintaining the level above 30 per cent throughout the potential bleeding period, which is at least 10 days and may be considerably longer. If this control can be achieved, it is possible to perform most operations on hæmophiliacs with comparative safety (France & Wolf, 1965; Biggs & Macfarlane, 1966). In the case of an emergency, delay while obtaining



adequate laboratory assistance, is less risky than operating without sufficient precautions.

### **General Management of the Young Hæmophiliac**

As in many fields, it is necessary to adjust our attitudes to changes brought about by technical advances. Hæmophilia is no longer inevitably crippling and severe forms are no longer likely to be lethal. In pædiatrics a very special approach is needed. Infusing small children requires great skill. However, tiding patients over acute episodes is not the only problem. Mothers must be advised about home-care and the importance of bringing the child for treatment at the onset of hæmorrhage. Co-ordination with School Medical Officers should be routine as the education of hæmophilic boys presents a most difficult problem. These boys are intellectually normal and should, therefore, be educated with others of normal intelligence. However, even though they may not become cripples, they are not suited to occupations involving physical strain and for them, more than for healthy boys, long stretches of missed schooling can be disastrous. Recent surveys suggest that the only satisfactory solution lies in the establishment of special boarding-schools, or special school units working hand-in-hand with day-treatment and hospital Hæmophilia Centres (Britten *et al.*, 1966). The Lord Mayor Treloar College, Froyle, near Alton, Hampshire, is at present the only boarding-school in England which really caters, both medically and educationally, for the hæmophilic boy. In January 1967 there were over 30 such boys attending this school. Although physically disabled, all pupils are of normal intelligence; this is not so at other schools for the physically handicapped.

Psychological maladjustment (almost always aggravated by medical mismanagement) can lead to one of two extremes, severe defeatism on the one hand and aggressive disregard of physical limitations on the other. There is now a growing awareness that it is often the docile patient, belonging to the first group, who is most in need of psychiatric help (Alby, Alby & Caen, 1962).

### **Hæmophilia Centres**

The Ministry of Health is now responsible for a network of Hæmophilia Centres, first established in 1954 by the Medical Research Council. Originally, the scheme offered little therapeutically, the aim being that the Centres should provide a laboratory service through which patients could be reliably diagnosed, registered and issued with official Hæmophilia Cards. Subsequently several of these Centres have developed highly efficient therapeutic services.

The first requirement for such a Centre is a hæmatologist, with specialized knowledge of blood coagulation, to train staff and supervise the laboratory aspects of the control of hæmorrhage. In addition he must be able to co-ordinate an appropriate clinical team. Both laboratory and clinical facilities must be available on a 24-hour basis. The importance of these points has been stressed by the President of the Hæmophilia Society, and other members of its committee (Dalrymple-Champneys, Hunter & Polton, 1967), who point out that, if a local hospital undertakes the management of a hæmophiliac,

it will always be essential for it to work in close co-operation with the Centre at which its patient is registered.

### OTHER CONGENITAL DEFECTS

Much of what has been said about hæmophilia, especially with regard to management, is applicable to other coagulation disorders. Compared to hæmophilia they are all uncommon. Factor VIII deficiency is also encountered as a life-long bleeding disorder in von Willebrand's disease and, rarely, in association with other specific factor deficiencies such as factor V. Von-Willebrand's disease will be considered next although it could as well be classified with conditions associated with a long bleeding time.

#### Von Willebrand's Disease

Von Willebrand's disease is a bleeding tendency which is inherited as an autosomal dominant trait, and characterized by a prolonged bleeding time and a deficiency of factor VIII.

*Hæmatological Findings.* The patients originally investigated by von Willebrand (1926, 1931) were inhabitants of the Åland Islands off the Finnish coast. The hæmatological diagnosis was based entirely on their prolonged bleeding time. In 1953 the association of a prolonged bleeding time with a deficiency of factor VIII was reported both by Alexander and Goldstein and by Larrieu and Soulier. Re-investigation of von Willebrand's original patients, or their descendants, was independently carried out in 1957 by Nilssen and co-workers and by Jurgens and co-workers, who confirmed the associated factor VIII deficiency. The characteristic combination of the two abnormalities has now been recognized in most countries.

In some important ways the factor VIII deficiency in von Willebrand's disease differs from the factor VIII deficiency in hæmophilia. In contrast to the characteristically uniform factor VIII level in members of the same family affected with hæmophilia, in von Willebrand's disease the level of factor VIII commonly varies from one affected member of the family to the next. Furthermore, the response to infusions of various plasma preparations is different in the two diseases. In hæmophiliacs the rise and fall in factor VIII level after infusions of normal plasma, human AHF or cryoprecipitate, can be interpreted in terms of the amount of factor VIII infused and its decay; the maximum concentration of factor VIII occurs immediately after the infusion, after which it immediately starts to fall. In patients with von Willebrand's disease, in contrast, the initial rise in factor VIII as in the case of hæmophilia reflects the factor VIII content of the preparation given; but, instead of gradually declining the level continues to rise for some hours after the infusion has been discontinued; the period of decay is also protracted. Similarly, a delayed rise in the factor VIII level of patients with von Willebrand's disease can be obtained by infusing hæmophilic plasma or certain other blood products deficient in factor VIII, e.g. fibrinogen prepared from time-expired bank blood (Biggs & Matthews, 1963). The reverse is not true—i.e. the hæmophilia defect cannot be corrected by either von Willebrand plasma or fibrinogen; nor does the *in vitro* mixing of hæmophilic

and von Willebrand plasma cause a rise in factor VIII activity. These findings suggest that the synthesis of factor VIII which occurs in von Willebrand's disease is a cellular process. One explanation offered for this intriguing phenomenon is that factor VIII has two precursors and that the presence of the abnormal von Willebrand factor leaves an excess of normal chains able to combine with their opposite pair in hæmophilic or normal plasma. Reciprocal correction is not possible because the hæmophilic would not have the correct polypeptide available to combine with the normal chains available in the von Willebrand plasma (Graham, 1965). Blombäck, Jorpes & Nilsson (1963), noting that the infusion of hæmophilic plasma into patients with von Willebrand's disease shortened the bleeding time, suggested that the primary genetic defect may involve a yet unidentified anti-bleeding factor which influences both the vascular response to injury and the synthesis of factor VIII.

The contradictory views which have been expressed about the presence or absence of a distinct platelet abnormality in von Willebrand's disease have their roots in the multiplicity of platelet-function tests. By performing serial platelet counts during a prolonged bleeding time test, Borchgrevink (1960, 1961) has inferred that the platelets of people with von Willebrand's disease do not stick to damaged tissue in the normal way. Salzman (1963) aspirated blood directly from a vein, and through a column of special grade glass beads into a vacutainer. He counted the proportion of platelets lost in the column and demonstrated a significant reduction in the adhesiveness of platelets in von Willebrand's disease. As this procedure is difficult to standardize there has been much controversy about these findings, though several workers (e.g. Strauss, Bloom & Butts, 1965; Dormandy, 1967; Meyer *et al.*, 1967; O'Brien & Heywood, 1967) claim to have confirmed them. Platelet aggregation by adenosine diphosphate and the release of platelet thromboplastin (platelet factor 3) are normal in von Willebrand's disease.

*Clinical Features.* Patients with mild von Willebrand's disease have a characteristic tendency to prolonged superficial bleeding after minor cuts and injuries; menorrhagia and bleeding from mucosal surfaces (gums, nasal mucosa) are common. In more severe cases these complaints are overshadowed by the types of bleeding characteristic of factor VIII deficiency—large hæmatomas, profuse and prolonged post-operative hæmorrhages, and spontaneous hæmarthroses. In the mild cases there may be long spells when the bleeding time is normal or near-normal, and the test may have to be repeated at intervals. In neither group can the diagnosis be made without a factor VIII assay. In mild cases the concentration may be in the low-normal rather than the frankly abnormal range; but, in conjunction with the family and clinical history, it usually permits a presumptive diagnosis. Investigation of patients' relatives may bring unsuspected cases to light.

Treatment which corrects the factor VIII deficiency will usually control hæmorrhage even if the bleeding time remains prolonged (Biggs & Matthews, 1963). Infusions of fresh or fresh-frozen plasma are generally adequate. Since treatment results in a greater rise of factor VIII than would be expected in the case of hæmophilia, it is usually adequate to give infusions on alternate days. Concentrates of human anti-hæmophilic factor such as HAHF (prepared by the method of Kekwick and Wolf), the Blombäck fraction I-O,

or Pool cryoprecipitate all contain the von Willebrand factor and can be used for the control of major hæmorrhagic episodes.

### Factor IX Deficiency

Clinically and in its hereditary pattern factor IX deficiency (Christmas disease, Hæmophilia B) is indistinguishable from hæmophilia. Tests for diagnosis are based on the same principle as those for hæmophilia, i.e. the prothrombin time is normal and the thromboplastin time prolonged; this abnormality is corrected by normal and by hæmophilic plasma, but not by factor IX deficient plasma. A serum defect, with a normal plasma component, is found in the thromboplastin generation test; once again the diagnosis ultimately rests on the specific factor assay.

Factor IX is much more stable *in vitro* than factor VIII; so the patient's circulatory capacity is the main limitation of treatment. Concentrate is produced in this country only at the Oxford Hæmophilia Centre (Biggs *et al.*, 1961, 1966) and, on a small scale, at the S.E. Scotland Transfusion Centre.

Two brothers, in whom an inhibitor causing prolongation of the one-stage prothrombin time was associated with a factor IX deficiency, have been described by Hougie & Twomey (1967) as a new type of factor IX deficiency; family studies are reported and the genetic significance discussed.

### Factor II (Prothrombin), Factor VII and Factor X Deficiencies

An isolated deficiency of any of these factors is always genetically determined and the inheritance of each appears to be autosomal recessive.

Factor X deficiency is extremely rare: Bachmann (1958) listed details of 19 patients from 12 families in whom the diagnosis had been unequivocally established. Hæmorrhagic manifestations are seldom severe, although hæmarthroses have been recorded. Heterozygotes may show a partial factor deficiency.

Factor VII deficiency is also rare (Owen *et al.*, 1964; Marder & Shulman, 1964). Abnormal bleeding occurs in homozygotes who have factor VII levels of less than 10 per cent. Heterozygotes are clinically normal but have factor VII levels of around 50 per cent.

Only a few well-authenticated cases of factor II (prothrombin) deficiency have been reported (Borchgrevink *et al.*, 1959; Pool, Desai & Kropatkin, 1962). Many patients, previously reported to be deficient in this factor, have subsequently been proved to be cases of factor V or factor VII deficiency.

Factors VII and X are strikingly similar in their properties and constitute, together with factor IX, the stable serum factors which are not consumed in clotting. Factor II is included in the group because all four factors are vitamin-K-dependent and tend to become depleted in liver disease, in the new-born, and in patients treated with anticoagulants of the coumarin type. Deficiency of any member of this group will prolong the quick one-stage prothrombin time. The partial thromboplastin time, the stypven time, the two-stage prothrombin time and other special tests will help to distinguish between the separate deficiencies (Hardisty & Ingram, 1965; Biggs & Macfarlane, 1962).

Combined congenital deficiencies, closely resembling the effects of

coumarin treatment, have been described by Newcomb and co-workers (1956). At the opposite extreme, in the coumarin-resistant kindred of O'Reilly and co-workers (1965), seven members of a family in three generations showed extraordinary resistance to the prothrombopenic effects of the coumarin compounds, despite the normal absorption and metabolism of the drug. The propositus required 145 mg of warfarin daily (20 times the average dose) to maintain his prothrombin time in the therapeutic range.

### **Factor V Deficiency**

Factor V deficiency can be inherited either as an isolated congenital defect or in combination with a deficiency of factor VIII. The mode of inheritance is autosomal recessive. The first patient to be described with this defect (Owren, 1947) had an exceptionally severe bleeding disorder, although many of the subsequent cases have been relatively mild. Factor V is highly labile, and for this reason fresh or fresh-frozen plasma is required for correction of the defect.

### **Factor XI and Factor XII (Contact Factors)**

Factor XI and factor XII are both involved in the initial contact stage of intrinsic blood coagulation. Their properties have been reviewed by Nossel (1964). Factor XII deficiency does not result in a hæmorrhagic disorder, although *in vitro* the coagulation of blood from affected people is grossly abnormal. Factor XI deficiency appears to be largely confined to people of Jewish extraction (Rosenthal, 1955). It generally results in a mild hæmorrhagic state which becomes manifest after operation or severe injury (Rapaport *et al.*, 1961). Prophylactic control is easily achieved not only with fresh or fresh-frozen plasma but also with the supernatant plasma remaining as a by-product of the preparation of Pool factor-VIII-cryoprecipitate (Bennett & Dormandy, 1966). Both contact factor deficiencies are inherited as autosomal recessive genes.

### **Factor I (Fibrinogen)**

Fibrinogen is synthesized in the liver (Favre-Gilly, 1947), but little is known about its biosynthetic pathway. Its turnover rate is much slower than that of other clotting factors; the biological half-life of injected fibrinogen in the circulation is of the order of four days. Adequate concentrations can be maintained without difficulty in the plasma of an adult patient by weekly infusions of 15 g of fibrinogen (Ingram, Pinniger & Vallet, 1960).

**Qualitative Defects.** The fibrinogen molecule is species-specific; amino acid studies suggest that the site of the proteolytic reaction, catalysed by thrombin, differs in man and also in animals (Clegg & Bailey, 1962; Doolittle & Blombäck, 1964; Blombäck & Yamashina, 1958). The fibrinogen variants described by Ménaché (1964) and by Beck and co-workers (1965) may differ structurally from normal fibrinogen, just as foetal and abnormal hæmoglobins deviate from normal adult hæmoglobin. The clinical consequences of such defects may depend on whether or not they involve the amino acid sequences

attacked by thrombin. Affected members of the family described by Ménaché were clinically normal, while those studied by Beck and co-workers suffered from a definite bleeding disorder.

*Quantitative Defects.* More than 60 cases of congenital afibrinogenæmia have been described. No plasma fibrinogen can be detected in such cases by conventional analysis, but (as with agammaglobulinæmia and analbuminæmia) traces are often demonstrable, using very sensitive immunological techniques. The hæmorrhagic symptoms are surprisingly mild; spontaneous hæmorrhage and hæmarthroses are rare, and it is only after accident or surgery that hæmorrhagic symptoms become manifest. Nevertheless, among the cases described there were 14 deaths from hæmorrhage and 16 undiagnosed younger siblings had died as infants from umbilical bleeding (Kerr, 1965). The mode of inheritance is autosomal recessive.

The prevalence of congenital hypofibrinogenæmia is more difficult to assess. The normal plasma fibrinogen concentration has been given by Biggs and co-workers (1962) as 250–400 mg/100 ml, but the true range may be even wider. Incomplete documentation raises doubts as to whether many of the reported cases of inherited partial deficiencies may in fact have been acquired secondary depletions. However, a number of well-authenticated cases with fibrinogen levels of less than 70 mg/100 ml do appear to have been genetically determined (Kerr, 1965).

### Factor XIII

Unlike most other clotting factors, the fibrin stabilizing factor was recognized at least 15 years before a case of the congenital deficiency was diagnosed (Robbins, 1944; Laki & Lorand, 1948). Clots formed by the recalcification of normal plasma were shown to remain stable when put into 5 M urea, whereas fibrin resulting from the action of thrombin on purified fibrinogen was dissolved. The plasma factor apparently responsible for the stability of the clot was then known as fibrin stabilizing factor and only later was it designated factor XIII. In 1960 Duckert, Jung & Shmerling reported a family in which they recognized a deficiency of fibrin stabilizing factor in four members suffering from a hæmorrhagic diathesis. Since then several other cases, including two from England (Lowsowsky, 1965), have been reported. The subject is reviewed by Duckert (1965).

As all test systems, up to the stage of thrombin formation, are normal, factor XIII deficiency must be looked for specifically whenever the clinical symptoms are suggestive. The severity of the hæmorrhagic diathesis is variable. Umbilical bleeding is typical and deep hæmatomata are not unusual, but only two of the patients described sustained hæmarthroses. Thin papery scars, the development of cord stumps due to abnormal fibroblast development (Beck, Duckert & Ernst, 1961), and defective wound healing are among the clinical characteristics. Prophylaxis and treatment are by infusion of fresh-frozen or even dried plasma. Like fibrinogen, the half-life of factor XIII is of the order of 4 days and the effect of infusions lasts for 10–14 days. The hereditary pattern is autosomal and incompletely recessive.

Preliminary investigations involve testing the solubility of recalcified plasma in 5 M urea. The clot from a patient deficient in factor XIII will dissolve within 24 hours; a normal clot will not.

### ACQUIRED COAGULATION DEFECTS

Acquired coagulation defects can arise either as a result of defective synthesis of the clotting factors or secondary to the appearance of active inhibitors or inactivators. In liver disease, for example, the synthesis of several factors may be depressed. In the "defibrination syndrome", on the other hand, normal synthesis cannot keep pace with excessive destruction.

Acquired coagulation defects have been recognized in many diseases in recent years. Only the most common syndromes will be considered here.

#### Liver Disease and Vitamin K Deficiency

The bleeding states associated with vitamin K deficiency and liver disease are best considered together. Vitamin K is an essential precursor of four clotting factors—factor II (prothrombin), factor VII, factor IX and factor X, all of which are synthesized by the liver (Ratnoff, 1960, 1963; Sherlock *et al.*, 1961). Because of the multiplicity of vitamin-K-dependent factors, the associated bleeding tendency is less predictable clinically than in inborn bleeding defects, and laboratory tests can rarely establish the nature of the defect with the same degree of accuracy. The most useful single test—both in diagnosis and as a prognostic index—is the one-stage prothrombin time.

Liver disease can give rise in at least two ways to deficiencies of Vitamin-K-dependent clotting factors. Obstructive jaundice tends to impair the absorption of the vitamin and parenchymatous failure impairs its utilization. The two mechanisms are not always separable either on clinical grounds or by laboratory procedures (Sherlock *et al.*, 1961). Nevertheless, in conjunction with other liver-function tests, the one-stage prothrombin time can give a diagnostic lead. Shortening of the prothrombin time after administration of vitamin K suggests biliary obstruction, whereas lack of response points to primary hepatocellular failure (Owren, 1949).

**Management.** Because of the various ways in which hepatic disease predisposes to bleeding, the investigation of these patients for liver biopsy, or pre-operatively, must include at least three hæmatological tests—a prothrombin time, a platelet count and, if the platelet count is low, a bleeding time. If the use of inhibitors to fibrinolysis is contemplated, tests for fibrinolytic activity must also be included.

A prolonged prothrombin time calls for vitamin K therapy. Treatment should not be abandoned, because of failure to respond, before a course of 50 mg daily has been given intravenously over a period of 3–4 successive days (Hardisty & Ingram, 1965). If there is no response, fresh citrated plasma or fresh-frozen plasma (15–25 ml/kg body-weight) should be administered by rapid intravenous infusion. The two-stage prothrombin assay is a useful guide in severe cases.

The causes of thrombocytopenia in liver disease are obscure and probably multiple (Cohen, Gardner & Barnett, 1961). A pre-operative platelet count of less than 50,000/cmm, especially if it is associated with a prolonged bleeding time, is a reasonable indication for a concentrated platelet transfusion (see p. 89).

In cases of severe hepatic failure, or as a preliminary to major operations, several other investigations may be necessary. Factor V deficiency is a well-

recognized complication of advanced liver-cell damage and can be excluded only by specific assay. The diagnosis may be important because (unlike factor VII and factor X deficiency) it cannot be adequately treated by bank blood. A low circulating fibrinogen (factor I) level may reflect not only a lowered rate of synthesis but also an active fibrinolysis (Grossi, Morens & Rousselot, 1961; von Kaulla, 1964). One of the anti-fibrinolytic agents discussed below may prove valuable if excessive fibrinolysis is demonstrable; but the risks involved are not yet sufficiently clear to justify their indiscriminate prophylactic use.

In the early inflammatory phase of liver disease the plasma fibrinogen may be raised, but this is not generally associated with abnormal coagulation. However, three cirrhotic patients with hypercoagulability, fibrinolysis and bleeding were described by Zetterqvist & von Francken (1963). In each of these the most striking laboratory finding was a grossly raised factor VIII level. Bleeding was controlled and the factor VIII level restored to normal by intravenous heparin. Activated factor VIII (VIIIa), occurring as an unusual manifestation of intravascular coagulation, presumably accounted for its very high initial level.

#### **Intravascular Coagulation and the Defibrination Syndrome**

The defibrination syndrome includes several abnormal mechanisms which, singly or in combination, precipitate an acute and sometimes catastrophic hæmorrhagic state. The syndrome is almost always associated with abnormal intravascular coagulation and in its severe form is characterized by the large-scale destruction of platelets and the massive consumption of clotting factors. It seems that abnormal clot-lysis is not usually the main cause of the bleeding, though activation of the fibrinolytic mechanism is common.

Intravascular coagulation and defibrination is most often seen as an acute obstetrical emergency, complicating concealed or accidental hæmorrhage, retention of a dead foetus, amniotic-fluid embolism, or septic abortion. It can follow surgical trauma, especially the handling of the thoracic and pelvic viscera (e.g. prostatectomy), and procedures requiring cardio-pulmonary by-pass. It is a recognized, though rare, complication of disseminated carcinoma, pancreatitis, liver disease, snake-bite and drug-sensitivity reactions. A residue of cases in which no primary cause is evident constitutes the idiopathic group. Minor degrees of defibrination are not uncommon: they require no treatment.

Whatever the underlying cause, the defibrination syndrome can be immediately life-threatening and every laboratory should, therefore, be equipped to provide prompt diagnostic and therapeutic guidance. Detailed plans for the clinical and laboratory approach to such a problem are well set out by Hardisty & Ingram (1966). It must not be forgotten that an acute bleeding state, clinically indistinguishable from defibrination, is sometimes due to a previously unsuspected long-standing coagulation defect. If three blood samples are taken from the start, into sequestrene, citrate and citrate-EACA (epsilon aminocaproic acid) solution, it should be possible to obtain a presumptive diagnosis within one hour. The initial tests should include the fibrinogen titre, the thrombin time and the platelet count. Fibrinolytic activity can be assessed simultaneously by comparing the fibrinogen titre



estimated with the citrate and citrate-EACA samples of plasma (Sharp & Eggleton, 1963).

The fibrinogen titre is measured by adding an optimal concentration of thrombin to doubling dilutions of plasma. The dilution at which the patient's plasma clots, in comparison with that of the control, indicates the amount of fibrinogen present. In the thrombin time test, a prolongation of the clotting time of the plasma, measured after the addition of thrombin, indicates a deficiency of fibrinogen or interference with the thrombin-fibrinogen reaction.

A normal fibrinogen titre and thrombin time, in a patient with an acute bleeding state, suggests an inborn coagulation defect and further tests should be appropriately directed along these lines. If either test is abnormal (and heparinæmia has been excluded) a presumptive diagnosis of defibrination can be made. Associated thrombocytopenia is to be expected. Fibrinogen estimations and further specific assays are then necessary to assess, as far as possible, the relative importance and severity of the multiple depletions responsible for the bleeding.

The management of each patient must be based on laboratory findings. Most patients who present as acute bleeding emergencies will already have had several pints of blood. If defibrination is confirmed, an immediate infusion of 6 g fibrinogen in 200–300 ml distilled water can be life-saving, but, when the consumption of clotting factors is progressive, the effect is transient. If an emergency operation, e.g. a Cæsarean section, is imminent or in progress, a platelet count of less than 100,000/cmm may call for an immediate platelet infusion. Factor V and factor VIII depletion cannot be corrected by bank blood and fresh or fresh-frozen plasma must be used. In all cases every effort must be made to eliminate the precipitating cause; the speedy completion of the delivery or operation will often reverse the bleeding abnormality. When appropriate replacement therapy fails, an attempt must be made to arrest the underlying abnormal intravascular clotting by treatment with heparin.

### **Heparin in the Treatment of the Defibrination Syndrome**

The use of a powerful anticoagulant in the management of acute bleeding states may seem paradoxical, but the value of heparin is now well established. It is probable that the drug owes its effectiveness partly to its direct inhibitory action on clotting, but experimental evidence suggests that other mechanisms are also involved. In this connection the current revival of clinical interest in the Shwartzman phenomenon is relevant (Hjort & Rapaport, 1965; Rodriguez-Erdman, 1965; Hardisty & Ingram, 1966; Hardaway, 1966).

In a classical series of experiments, Shwartzman (1937) showed that an immunological hæmorrhagic response could be elicited in animals by two consecutive doses of Gram-negative endotoxins; the nature and severity of the response depended on the route of administration. When the initial subcutaneous injection was followed by an intravenous dose, a local hæmorrhagic lesion developed at the site of the first injection. Two intravenous doses, on the other hand, led to widespread parenchymatous hæmorrhages with bilateral renal cortical necrosis. In 1953 Kane, Good and Thomas demonstrated that the hæmorrhagic fibrinolytic element in the Shwartzman phenomenon could be prevented by heparin; and there is now substantial

evidence to show that this phenomenon is accompanied by intravenous coagulation and defibrination (Rodriguez-Erdmann, 1964). More recently, a number of workers have drawn parallels between the hæmorrhagic manifestations of the Schwartzman phenomenon and those arising in pregnancy septicæmia, various non-septic obstetrical emergencies, e.g. premature separation of the placenta and in meningococcal and other septicæmias of infancy (McKay & Wahle, 1955; Shumway & Miller, 1957; Kunzer & Aalam, 1964; Kibel & Barnard, 1964). These observations have led to the growing realization that the Schwartzman phenomenon, or something very like it, can and does occur in man. The generalized form may be involved in various hæmolytic uræmic syndromes, especially in children; and the cutaneous form in purpura gangrenosa or purpura fulminans. In the latter syndrome the coalescent purpura is usually accompanied, or preceded, by severe pain in the affected area—a factor that helps to differentiate this condition from bruising, or hæmatoma forming as the result of a defect of the hæmostatic or coagulation mechanism (Sharp, 1964).

The therapeutic value of intravenous infusions of heparin is now generally accepted. The underlying immune mechanism of the Schwartzman reaction, however, is far from clear; experimental evidence suggests that the cells of the reticulo-endothelial system become unable to clear plasma of substances which are able to initiate intravascular coagulation (Spaet *et al.*, 1961; Margaretten, Zunker & McKay, 1964).

### Therapeutic Inhibitors of Fibrinolysis

Antifibrinolytic agents are only indicated in the defibrination syndrome when excessive fibrinolysis, demonstrated by laboratory tests, is associated with hæmorrhage. It is important to emphasize that heparin cover should always be given at the same time because of the associated intravascular coagulation. Antifibrinolytic drugs act either by neutralizing the fibrinolytic enzyme, plasminogen, or by blocking its activation.

Antifibrinolytic drugs have been prepared both biologically and synthetically. Among the natural products, a bovine lung extract is available commercially under the trade name "Trasylol". Apart from inhibiting various proteolytic enzymes, including trypsin and pepsin, Trasylol has also got antifibrinolytic properties. *In vitro*, it also inhibits the coagulation mechanism (Amris, 1964) and the possibility that the drug was both antifibrinolytic and anticoagulant has been suggested. However, Gormsen & Josephsen (1967) failed to show any anticoagulant effect in their *in vivo* experiments. At present, the antifibrinolytic agent of choice is the synthetic compound, epsilon-aminocaproic acid (EACA), or its more potent isomer, para-aminomethylcyclohexane carboxylic acid (AMCHA) (Okamoto, 1959; Okamoto & Okamoto, 1962). Both compounds prevent fibrinolysis by inhibiting the activation of plasminogen (Alkjaersig, Fletcher & Sherry, 1959). A satisfactory blood concentration can be achieved by giving EACA orally (0.1 g/kg body-weight) every four hours, or 15 ml 10 per cent solution intravenously every hour. For the treatment of post-prostatectomy hæmorrhage effective levels are obtained with less frequent and smaller doses.

Antifibrinolytic therapy is not without risk. The drug is incorporated into the clot which will tend to resist physiological lysis. This accounts for such

complications as venous and arterial thromboses and the rapid development of constrictive or obstructive lesions, when the drug is used after bleeding into a visceral cavity. In a case reported by McNicol (1962) a patient, whose bleeding state after an open-heart operation was successfully corrected by EACA, died 36 hours later with a clotted hæmopericardium and hæmothorax. Other s.de-effects, less marked with AMCHA than with EACA, include hypotension, dizziness, diarrhoea and abdominal discomfort.

Despite these risks, antifibrinolytic agents have been useful in at least three groups of generalized fibrinolysis:—in the control of post-operative fibrinolytic bleeding in patients with advanced liver disease (Grossi, Moreno & Rousselot, 1961), in cases of overdosage with thrombolytic drugs, and in certain fibrinolytic states associated with the defibrination syndrome. Laboratory control is always essential.

In the control of local fibrinolytic activity, post-prostatectomy hæmorrhage has been successfully controlled with EACA. In the opinion of some workers (McNicol *et al.*, 1961; Douglas & McNicol, 1964), the danger of thrombosis prohibits the routine prophylactic use of the drug. Others, however, have not found any significant complication (Vinnicombe & Shuttleworth, 1966). EACA and AMCHA have also been shown to have a place in the control of menorrhagia, in women in whom uterine cancer has been excluded (Nilsson & Bjorkman, 1965; Andersson *et al.*, 1965). Cooksey, Perry & Raper (1966) report encouraging results from giving EACA to patients with hæmophilia and Christmas disease when undergoing dental surgery; further trials need to be carried out in order to assess their claims.

#### Naturally Occurring Acquired Anticoagulants

Naturally occurring anticoagulants which *block* the reaction between two clotting factors are known as inhibitors; those which *destroy* clotting factors as inactivators. Apart from those which develop after treatment in patients with hereditary coagulation defects, they arise in people whose hæmostatic mechanism had previously been normal. Laboratory diagnosis depends on showing that the patient's blood will inhibit or inactivate the clotting powers of a normal blood sample when they are mixed. Various test systems can be used. Broadly speaking, an inhibitor is present if a sample of normal blood or plasma, when combined with one of equal volume from the patient, fails to reduce the clotting time of the mixture to less than half of the difference between the clotting times of the individual samples. Inactivators may not be immediately apparent, but show a progressively increasing abnormality after incubation.

Heparin and heparin-like substances (inhibitors) have been observed in various chronic illnesses, including systemic lupus erythematosus and chronic nephritis, and also in otherwise healthy persons. The thrombin time, corrected by the addition of protamine sulphate or toluidine blue, is the most sensitive test for detecting the presence of heparin. Although failure of protamine sulphate to correct a prolonged thrombin time will exclude heparinæmia, it must be remembered that partial correction will often be obtained in the presence of fibrin breakdown products, as found in intra-vascular coagulation, etc. Speer and co-workers (1955) report the use of oral

toluidine blue in the management of one case; this drug, however, is unsuitable for long-term treatment since it produces methæmoglobinæmia (Salzman & Britten, 1965). Cortisone has been successfully given for the long-term treatment of two patients (Favre-Gilly *et al.*, 1958; Quick & Hussey, 1957).

*Inhibitors in systemic lupus erythematosus* were found in 12 per cent of 107 patients with this condition, tested by Margolius (1961). Occasionally a coagulation abnormality may be the first sign of this condition, for the prothrombin deficiency and inhibitor, in one of the patients studied by Biggs & Denson (1964), were detected eight years before other signs of the disease appeared. The laboratory results show a prolongation of the clotting time of whole blood, the prothrombin time and partial thromboplastin time tests. The inhibitory effect of the patient's blood can be demonstrated in all these tests. The thromboplastin generation test and thromboplastin screening tests are normal. In most cases, the two-stage prothrombin assay gives very low results. Further investigation and discussion on the mode of action of this inhibitor is given by Breckenridge & Ratnoff (1963) and Biggs & Denson (1964).

Hæmorrhage, in patients with systemic lupus erythematosus, is more likely to be due to thrombocytopenia than to an inhibitor, even when one has been demonstrated. In some patients treated with steroids, the inhibitor has diminished or disappeared, but this does not mean that the presence of an inhibitor is an indication for steroid therapy. Steroid therapy should be used only if warranted by the general condition of the patient.

*Inactivators of the circulating clotting factors* occur in a small proportion of treated patients with hæmophilia and Christmas disease; even more rarely, they arise in patients with congenital deficiencies of other clotting factors. Since they are inevitably directed against the factor which is lacking in the patient, treatment of hæmorrhagic episodes is very difficult. Occasionally, inactivators of factor VIII occur in otherwise normal women between two and three months after delivery. In 6 of 13 such women, reviewed by Margolius (1961), the anticoagulant eventually disappeared after periods of time ranging from 6 months to 11 years. Nilsson, Skanse & Gydell (1958) reported a remission after treatment with ACTH, although this is usually unsuccessful. Transfusion, which will stimulate further antibody production, should be avoided as far as possible in these patients.

Anticoagulants, mainly directed against factor VIII, have been described in association with penicillin reactions, rheumatoid arthritis, pemphigus, chronic rheumatic heart disease, dermatitis herpetiformis and in normal people. So long as treatment can be avoided, the anticoagulant will tend to disappear. In the face of major hæmorrhage, washed packed cells should be given in preference to whole blood; a temporary effect, which may secure control of the bleeding, will probably be achieved by giving very high doses of concentrated human factor VIII or of animal AHG.

#### Administered Anticoagulants

Apart from a brief consideration of the coagulation problems involved in the use of an extra corporeal circulation, this chapter does not offer any scope

for the discussion of administered anticoagulants for which the reader is referred to Douglas (1962).

### THE EXTRACORPOREAL CIRCULATION

The introduction of extracorporeal circulation has been associated with disasters from uncontrollable operative or post-operative hæmorrhage. Theoretically, such cases—carefully selected and prepared for operation at comparative leisure—should be ideally suited to the prophylactic correction of any bleeding tendency, but the individual response remains notoriously unpredictable. Patients with severe laboratory abnormalities may not bleed at operation, while supposed non-bleeders may get out of control (Schmidt *et al.*, 1961; Salzman & Britten, 1965).

Diagnostic difficulties can be partly attributed to the variety of hæmostatic defects which tend to complicate the type of illness that requires surgery with extracorporeal circulation. Thrombocytopenia, fibrinogen deficiency and a prolonged one-stage prothrombin time are all relatively common in patients with cyanotic heart disease (Hartmann, 1952; Jackson, 1963). It may be impossible to decide how far associated polycythæmia, congestive liver failure, or pre-operative drug therapy are responsible for the hæmorrhage. Salzman and Britten have pointed out that latent thrombocytopenia, induced by pre-operative quinidine and diuretic therapy, occasionally becomes manifest only during or after the operation, and can seriously jeopardize recovery.

The term "factor deficiency" implies a decreased clotting activity on laboratory testing. Such decreased activity is not easy to interpret after the blood has been extensively manipulated by an extracorporeal pump system. The most common and most striking hæmostatic abnormality is an early and precipitous fall in the platelet count, but post-operative bleeding may also be associated with the disappearance of various factors, particularly factors I, II, V and VIII, the appearance of circulating anticoagulants and fibrinolysis. On the other hand, in spite of multiple abnormalities, post-operative bleeding does not necessarily occur. It is now customary to prime pumps with stored blood (not more than 4 days old) rather than with fresh, despite the absence of viable platelets and the lack of factors V and VIII.

Anticoagulation with heparin, of the patient and the blood which primes the pump, adds difficulties both to the management of the patient and to the interpretation of clotting test results. After the operation, heparin must be neutralized with protamine sulphate, and protamine itself has marked anti-coagulant properties (e.g. it inhibits thromboplastin generation and, in high concentrations, precipitates fibrinogen). Neutralization of heparin is checked, after the standard dose of protamine has been given, by carrying out a thrombin time. If this is still abnormally prolonged, a protamine sulphate titration is carried out and the extra dose required is calculated. If post-operative hæmorrhage occurs after the heparin has been neutralized, the development of a defibrination syndrome, or of fibrinolysis, must be considered and appropriately treated.

Regional heparinization is used in extracorporeal circuits in which only a small fraction of the blood volume is shunted outside the body and in which the flow rate is slow (e.g. extracorporeal renal dialysis). It requires a careful

balance between heparin, added to the blood leaving the patient, and protamine, added to the blood re-entering. The heparin level in the patient should be monitored by the thrombin time test and protamine sulphate titration. Regional heparinization is not used in cardio-pulmonary by-pass because of the high flow rates involved and the need to prevent intravascular coagulation (Saltzman & Britten, 1965).

### QUALITATIVE PLATELET DEFECTS

There has been a growing realization in recent years that the three physiological mechanisms on which hæmostasis depends are not linked in an orderly sequence; they overlap in time and influence each other. In particular, disintegrating platelets have been shown to release a phospholipid, now referred to as platelet factor 3, which greatly enhances the efficiency of the intrinsic clotting process (Troup *et al.*, 1961). Platelet factors are designated by Arabic numerals. A recent review of platelet function is given by Hardisty (1967).

Some of the ways in which platelets affect the vascular response and coagulation are shown in Fig. 3.5. Assessment of some of these functions is not always possible. Platelets carry a number of powerful physiological regulator substances in high concentrations and it is perhaps too easy to jump to the conclusion that the release of these compounds at the site of injury plays a

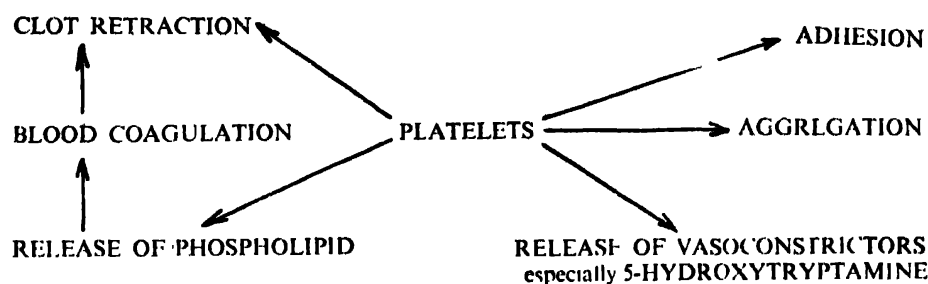


FIG. 3.5. The role of platelets in hæmostasis.

major defensive role. Platelets are particularly rich in 5-hydroxytryptamine, a strong peripheral vasoconstrictor, and many workers have suggested that the liberation of this substance by disintegrating platelets is a key event in hæmostasis. Against this view, it can be argued that reserpine therapy, which causes 5-hydroxytryptamine depletion, does not prolong the bleeding time nor does it induce abnormal bleeding (Shore *et al.*, 1956; Witte, Schricker & Schmid, 1961).

The effectiveness of platelets depends on their tendency to aggregate and adhere. In physiological hæmostasis these two functions are inseparable for, under normal conditions, platelets do not aggregate without coming into contact with an adhesive surface, nor do they adhere without aggregating. In platelet abnormalities, on the other hand, it is possible, and necessary, to study the two phenomena independently.

The discovery that catalytic amounts of adenosine diphosphate (ADP) induce rapid platelet aggregation (Gaarder *et al.*, 1961) has given impetus to much recent experimental work. ADP-induced platelet aggregation can be

observed directly under the microscope or measured as a change in the optical density of platelet-rich plasma (Born, 1962; O'Brien, 1962); the process requires  $\text{Ca}^{++}$  ions and can be inhibited by adenosine and various related compounds. ADP is identical with the platelet-aggregating substance liberated by lysed red cells (Hellem, 1960). Circumstantial evidence suggests that ADP plays an *in vivo* role similar to its *in vitro* action. The details of this action remain speculative.

Normal platelets readily adhere to any foreign surface and have a special affinity for collagen fibres (Zucker & Borrelli, 1962).

Great effort has been made in recent years to design a reliable laboratory test for measuring normal and abnormal platelet adhesiveness. Most of the methods depend on a comparison of the platelet count in blood or in platelet-rich plasma, before and after it has been passed over a glass surface. None of these procedures can yet be regarded as entirely satisfactory. Because the critical surface properties (e.g. charge and regularity, etc.) remain unknown, the conditions of exposure are difficult to standardize. A second difficulty is the relative inaccuracy of platelet counts on which the tests depend.

In addition to quantitative platelet defects (i.e. the primary and secondary thrombocytopenias), bleeding states can also be caused by platelets which are normal in number but abnormal in character. These *qualitative* defects can be congenital or acquired.

*Hereditary Qualitative Defects.* Glanzmann (1918) appears to have been the first to describe a hereditary disorder of qualitative platelet function and, since then, much terminological confusion has arisen because authors have described functional platelet defects under a variety of names. Despite many attempts to fit all inborn hæmorrhagic states into well-defined categories, the coagulation and platelet defects already mentioned leave a large and heterogeneous group unclassified. In the first instance, these patients usually present with a prolonged bleeding time and a normal platelet count; on further investigation, either their coagulation, the platelet function or their vascular response may come under suspicion. Von Willebrand's disease, which is previously discussed, and thrombasthenia are the only conditions in this group which can now be diagnosed on the basis of widely accepted genetic, hæmatological and clinical criteria.

Braunsteiner (1955, 1956) divided the functional platelet disorders into two groups, thrombasthenia and thrombopathy; many subsequent workers have followed this classification. The distinction, however, is by no means clear cut. *Thrombasthenia* is now recognized as a distinct clinical entity, inherited as an autosomal recessive character; affected people have a prolonged bleeding time but a normal platelet count. The diagnostic laboratory finding is that the abnormal platelets do not respond by aggregation, even to large concentrations of ADP, and without aggregation they seem unable to adhere to foreign surfaces or to discharge their thromboplastin function (Ulutin, 1961; Hardisty, Dormandy & Hutton, 1964). Conditions which would fit in with the term thrombopathy, in which both the platelet count and platelet aggregation with ADP is normal but the clot retraction abnormal, have been described (Kanska *et al.*, 1963; Alagille *et al.*, 1964). They are exceptionally rare and have not been universally accepted as definite entities. Hardisty & Hutton (1967b) have recently studied 13 patients, mostly with

mild hæmorrhagic disorders, in which normal initial aggregation was followed by rapid disaggregation.

**Acquired Qualitative Defects.** As with coagulation disorders, acquired qualitative platelet defects represent a more heterogeneous and less predictable group than that of the inborn abnormalities. For example, the bleeding tendency in uræmia can be attributed to the effect of toxic substances, which may both interfere with platelet function and depress the production of platelets by the bone marrow (Marcus & Zucker, 1965). Abnormal findings include reduced platelet adhesiveness, poor prothrombin consumption and an abnormal release of platelet factor 3. The finding that some uræmic plasmas inactivate normal platelets suggests that this is a secondary phenomenon (Camalane *et al.*, 1958). High molecular weight Dextran can also directly affect platelets or precipitate fibrinogen, which may cause their aggregation and subsequent disappearance (Ewald *et al.*, 1965).

Treatment of hæmorrhage, in patients with qualitative platelet defects, is the same as for thrombocytopenia. If whole blood is required, it should be taken into plastic blood-bags so that the platelet loss is minimized. If red cells are not needed, platelet-rich plasma or platelet concentrate is used. Although each platelet infusion for an adult is usually prepared from 5–6 pints of blood, it is unlikely to raise the platelet count by more than about 100,000/cmm.

The anticoagulant of choice for a platelet preparation is acid citrate dextrose (ACD) not sequestrene (EDTA) which has been used for years. The tendency for platelets to clump and aggregate when standard ACD is used can be overcome by lowering the pH of the anticoagulant or by increasing the citrate concentration. In order to avoid damaging the red cells by this increased citrate concentration, the extra citrate is added to the platelet-rich plasma after it has been separated (Mollison, 1967). By using the Fenwal ACD Platelet Pack, which has citrate in the satellite bag as well as in the blood-bag, the procedure is simplified and may be done entirely in a closed system.

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## CHAPTER 4

### POISONING

by

A. J. LEVI

#### MORBIDITY AND MORTALITY

THE incidence of poisoning in this country is rising and there is little evidence that the mortality is falling.

Poisoned patients usually die from cerebral anoxia or chest complications. Both often occur after the patient has entered a hospital and both are preventable. A reduction in mortality and morbidity can be achieved by regarding all poisonings as an emergency and by applying physiological and pharmacological principles in assessment and treatment.

In Great Britain as a whole there are no reliable figures for the mortality rate of poisoning. The Registrar General's figures (Reg. Gen. Statist. Register Eng. and Wales 1963) (Table I) show the enormous increase in actual number of deaths between 1953 and 1963 for both accidental and suicidal poisoning. The most common agents are "analgesic and soporific substances" which includes barbiturates and aspirin. The number of people dying from these substances each year is rising about six times faster than the cost of living. The increase in deaths from gassing is less striking. The total annual toll is over twice that of leukæmia and about the same as that from deaths on the road. Yet the problem receives neither the concern given to the cost of living, the intensive research efforts given to leukæmia nor the publicity given to road deaths.

TABLE 4.I

<i>Deaths Resulting From:</i>	<i>1953</i>	<i>1963</i>
Accidental and Suicidal use of analgesics and soporifics	915	2,505
" " " use of gas	2,857	3,768
Leukæmia	2,121	2,830

In a recent survey (Parkin & Stengel, 1965) on the incidence of suicidal attempts in Sheffield (a city of almost half a million people) it was found that the rate of such attempts to actual suicides was almost 10 : 1 over a two-year period. Almost one in five of these attempts were treated at home by their general practitioner. Unfortunately there is no record of the mortality on patients who were alive when first seen by a doctor; so the overall morbidity and mortality cannot be calculated.

The drugs used are commonly provided by physicians. Twelve per cent of the population take a sleeping pill at some time during the year. Many attempts are impulsive acts aimed at altering the life situation and not positive attempts to die (Kessel, 1965). Kessel found that more than one per thousand of the population of Edinburgh made such a gesture each year but

that 26 per cent of the men and 20 per cent of the women had no psychiatric illness. The vivid term "pseudocides" was coined by Lennard-Jones & Asher (1959) for these acts. Because many patients who survived did not have death as the purpose of the act Kessel prefers the term self poisoning. But reliance cannot be placed on the patient's judgement to take a sublethal dose. Nobody knows how many successful suicides did not mean to die.

The incidence of death from poisoning is increasing and as the doctors provide more complex and mixed preparations for ingestion so treatment becomes more difficult; prevention is impossible. Kessel (1965) sent a distraught weeping girl into six chemist shops in Edinburgh to buy 200 aspirins. No single bottle contains this number and few illnesses would need them, yet nowhere was she refused her request.

The treatment of poisoning has gone through various phases. Uncontrolled neglect was followed by an unfounded belief in stimulation. Then, controlled neglect—clearing the airway and letting the patient recover on his own—was followed by a resurgence of interest in newer and equally useless stimulants. The echoes of these are only just disappearing into the past. The current fashion in treatment is dialysis and diuresis. But forced diuresis is not the universal antidote and its value may become denigrated if it is widely misapplied.

The evolution of treatment has been documented most fully in Scandinavia (Clemmesen, 1963; Myschetzky, 1964; Ohlsson & Fristedt, 1962; Hagstam & Lindholm, 1964). Fig. 1 shows the mortality in cases admitted to hospital

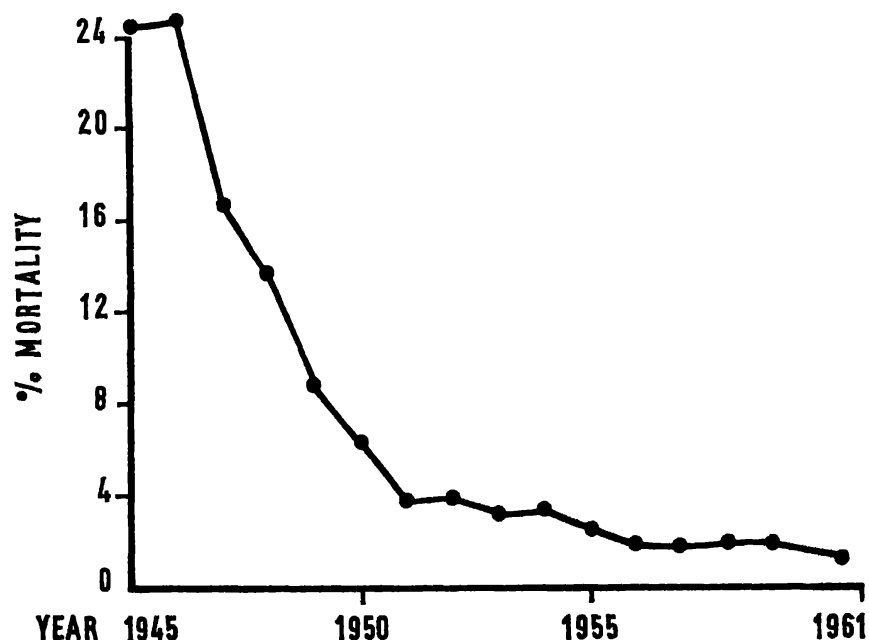


FIG. 4.1. Mortality in Cases of Poisoning admitted to Hospital. Denmark 1945-61.

in Denmark in recent years, it is below 1 per cent. When interpreting these figures it must be remembered that the very long acting barbiturates were the agents involved in most cases. These are largely excreted unchanged by the kidneys and are not commonly prescribed in this country. Recently, it has been

emphasised (Goulding, 1965) that the current trend is for poisoning to be by polypharmacy though there may be one main agent. This makes rational therapy more difficult. It also makes the question of tablet identification more urgent. It increases the demands on laboratories to provide data on the identity and blood levels of poisons 24 hours a day, 7 days a week. Few laboratories are able to undertake such a service even from 9 a.m. to 5 p.m., 5 days a week. This situation might be improved in special centres.

The prognosis in the severely poisoned patient may well be dependant on whether the right action is taken urgently when he first comes under medical care. The junior members of the hospital staff are often responsible, therefore, for decisions on treatment (or neglect) during these brief but violent illnesses.

### Poisons Information Centres

In 1953 the first poisons information centre in the world was started in America and 8 years later the voluntary bureau in Leeds was opened to the public. The Ministry of Health followed by opening 4 more operating from Guy's Hospital in London, the Royal Infirmarys in Edinburgh and Cardiff and the Royal Victoria Hospital, Belfast. An account of the first year's work of the National Poisons Information Service has now been published (Goulding & Watkin, 1965) and from Leeds has come an analysis of their first 3 years' work (Ellis & Blacow, 1965).

These centres apparently fill a need as about 500 calls a year are received by each one. Over half the calls are about children and a high proportion about domestic materials. Leeds is the only centre that accepts calls from relatives and finds this accounts for only 10 per cent of the work. They reduce the number of patients coming to hospital by being prepared to give reassurance over the telephone. The centre's functions include the accumulation of information on the toxicity of drugs and household products, and the effects of treatment. This information is made available to those who need it. The centres should help in the identification of an unknown ingested material, and publicize precautions to be taken in the home with potentially toxic substances.

Doctors who have consulted the Service are asked to give an account of the outcome of their patient in order to provide experience for future reference. The response rate is only about 60 per cent.

### Tablet Identification

Many attempts to classify or mark tablets and their containers have been made but none seems acceptable to all the interested parties: it has been suggested and is now official British Medical Association policy that all drug containers should be labelled with the name of the contents unless the prescriber specifically requests this is not to be done. Thus the right of the doctor *not* to tell his patient what treatment he is to receive is preserved. But this is not accepted practice. The agreed phrase—"Please label container with name of drug" is so much longer than the older instructions *nomen proprium* (or n.p.) or *recipe et signum* which the pharmacists have anyway been advised to ignore. Patients are liable to put tablets from one box into another whatever the label states.

Many schemes have been suggested. The two main possibilities, are either



indexing all marketed preparations centrally on a basis of physical characteristics (colour, size, shape, etc.) or systematically coding them with letters and numbers while inaccuracy and cost are the two main objections, to any particular scheme. Though desirable, a scheme of tablet identification is regarded as impracticable by the committee of Medical Science, Education and Research of the British Medical Association and by the Ministry of Health Standing Advisory Committee. However, a substantial attempt has been made by one manufacturer to produce a set of coded standard tablets (initially 169 from the B.N.F., B.P. and B.P.C.). This suggestion has been publicized by the Ministry of Health, condemned by the Pharmaceutical Society and rejected by the Joint Committee of the British Medical Association and the Pharmaceutical Society.

### RENAL EXCRETION AND METABOLISM OF DRUGS

At the turn of the century Overton (1895, 1899, 1902) put forward a hypothesis. He wrote: "It occurred to me quite early that all compounds which are easily soluble in ether, oils and similar substances (i.e. more soluble in these than in water) penetrate with greater speed through living tissue; whereas those compounds which are water soluble, rather than fat soluble, penetrate very slowly." He tested this hypothesis by doing over 10,000 experiments with 500 compounds in plants and then with 300 compounds in tadpoles. The relevance of this work to the absorption of foreign compounds from the gut and their excretion by the kidneys was unappreciated for many years.

Travell (1940) reported two experiments. He showed that when strychnine (a weak base) was injected in acid solution into the tied stomach of a cat it had no effect on the animal, but that when the injection was made in alkaline solution it was rapidly absorbed and the animal died. This demonstrated that the cell membrane in the stomach was more soluble to the unionized molecule than the ions. This phenomenon is now called non-ionic diffusion. The quantitative aspects of non-ionic diffusion were reviewed by Milne, Scribner & Crawford (1958). Many drugs are weak acids or bases therefore the proportion of ionized to unionized drug varies with the pH of the medium.

#### Renal Excretion

Renal excretion of many drugs appears to be mainly by a three mechanisms (Weiner & Mudge, 1964), glomerular filtration of the non-protein bound fraction, active proximal tubular secretion and passive pH dependent back diffusion in the distal tubule (see Fig. 4.2). A fourth mechanism, pH independent diffusion in the proximal and distal tubule, is of minor importance.

When a drug is mainly excreted by the kidney its plasma half-life is dependent on its volume of distribution and the renal clearance (Butler, 1958).

$$t_{\frac{1}{2}} \text{ (min)} = 2 \ln \frac{\text{Vol. of distribution in ml}}{\text{renal clearance ml/min}}$$

Where  $t_{\frac{1}{2}}$  is the half-life in minutes and  $2 \ln$  is the natural logarithm of two.

This formula is valid whether the bound or unbound plasma concentra-

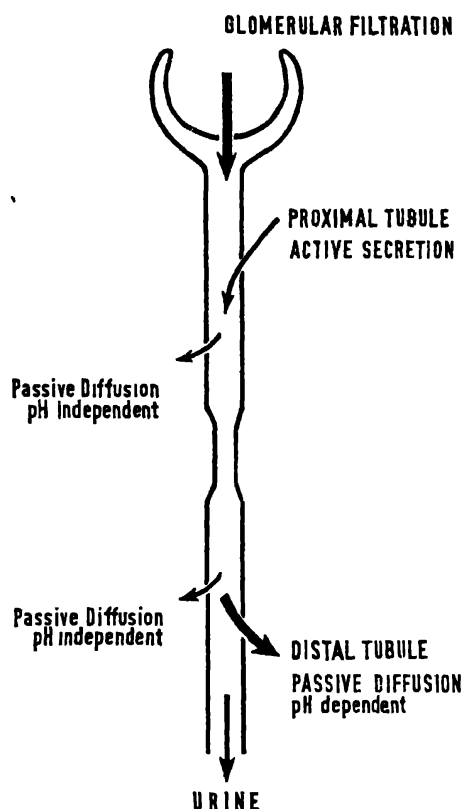


FIG. 4.2. Mechanisms of renal excretion of drugs.

tion is used provided the same figure is used for both. The half-life will be short if the volume of distribution is small (e.g. the extracellular fluid) and the clearance high (e.g. P.A.H.). It will be long if the volume of distribution is large (e.g. the total body water or if there is extensive protein or tissue binding) and the clearance is low, e.g. quinaquin or Teridax (iophenoxic acid, a radio opaque diagnostic agent). In the treatment of poisoning an important aim is to reduce the  $t_{1/2}$ . Little can be done to affect the volume of distribution but the renal clearance of many drugs may be considerably enhanced by manipulating the urine flow and pH. Also the effective clearance may be increased by use of a haemo or peritoneal dialysis.

### Glomerular Filtration

At the glomerulus only the non-protein bound fraction of the drug in the plasma will be filtered. Any factors which reduce the glomerular filtration rate such as hypotension or dehydration must reduce the filtered load. In contrast, correction of hypotension and dehydration or infusion of mannitol or bicarbonate may increase the glomerular filtration rate and thus the filtered load.

### Active Tubular Transport

In the proximal tubule there are two independent transport systems, one for organic acids and the other for bases. These active transport systems are remarkable for their lack of specific substrate requirements. Many organic acids, whether exogenous or endogenous, whatever the  $pK_a$  or structural

formula may be secreted into the tubule. If two substrates present simultaneously and both are, for instance, weak acids, the secretion of one may be blocked, e.g. the secretion of penicillin is blocked by probenecid. It is probable that the ionized fraction rather than the unionized is actively secreted. Protein binding does not limit secretion as dissociation is rapid. For instance, P.A.H. or penicillin clearance is almost complete (i.e. equal to renal plasma flow) despite significant protein binding. Competition, however, may limit secretion and could be an unsuspected effect if penicillin, acetazolamide, chlorothiazide or other therapeutic agent is given to the poisoned subject. The quantitative importance of these factors is unknown.

### Non-ionic Diffusion

Milne's detailed review of non-ionic diffusion remains the classic account of this physico-chemical phenomenon (Milne *et al.*, 1958). The distal renal tubule acts as a lipid membrane. Most drugs are weak acids or bases and in solution will be partly ionized. The proportion of ionized to non-ionized

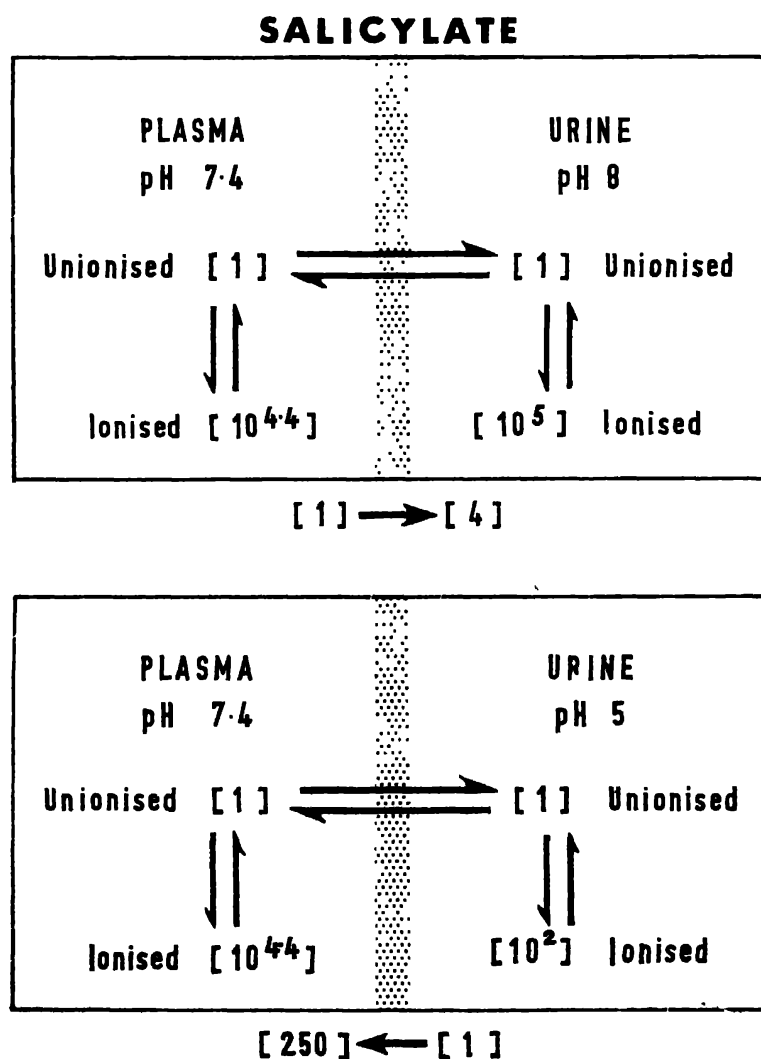


FIG. 4.3a. Distribution of salicylate between plasma and acid or alkaline urine.

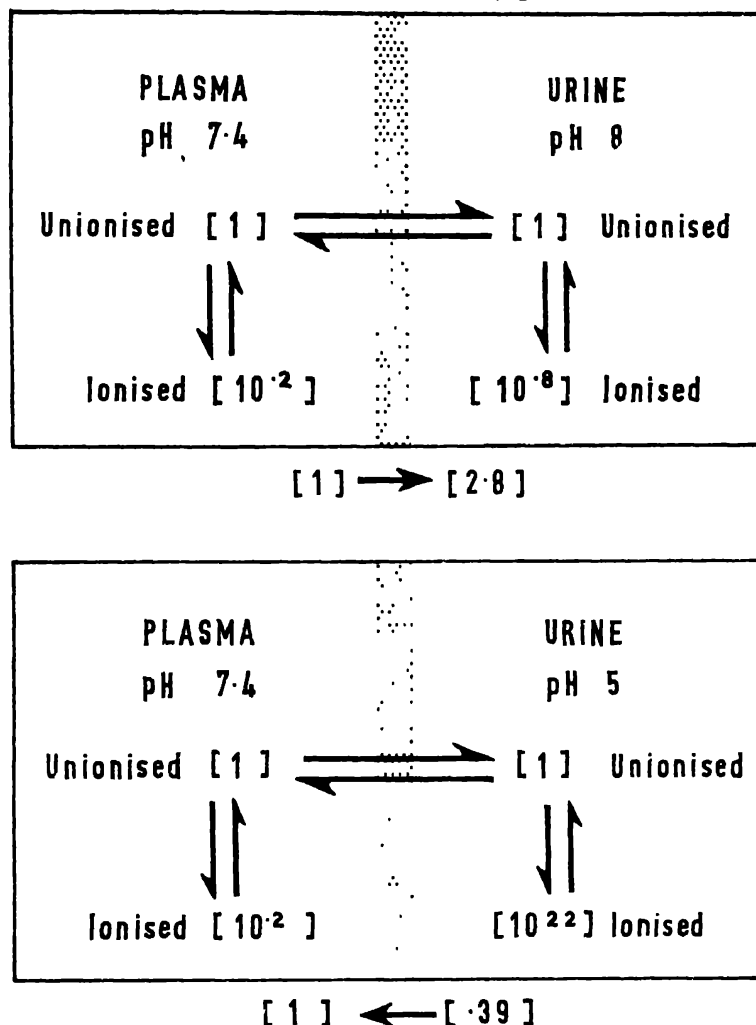
**PHENOBARBITONE**

FIG. 4.3b. Distribution of phenobarbitone between plasma and acid or alkaline urine.

drug in a solution at a particular pH may be calculated from the following formula:

$$\text{For an acid } \log \frac{I}{U} = \text{pH} - \text{pKa}.$$

$$\text{For a base } \log \frac{U}{I} = \text{pH} - \text{pKa}.$$

where I = concentration of ionized drug,

U = concentration of unionized drug,

pKa is the negative logarithm of the dissociation constant.

When  $\text{pH} = \text{pKa}$ , then  $I = U$ , i.e. the concentration of the ionized and unionized fractions are equal, and for a weak acid if the pH in the solution is higher than the pKa then more of the drug is ionized than unionized. Usually the unionized drug is relatively lipid soluble and diffuses freely across a lipid membrane such as the distal tubule, the stomach wall, the small intestine or the blood brain barrier. The ionized portion is lipid insoluble and thus

relatively impermeable to such a lipid membrane. If there is a hydrogen ion gradient between the two sides of the membrane then large concentration gradients of the drug may occur (see Fig. 4.3a, b).

The main factors affecting the concentration gradient are the pH difference and the dissociation constant (pKa) of the drug.

In fact the theoretical concentration gradients are not achieved for various reasons. Lipid membranes are not completely impermeable to the ionized component. Equilibration is often incomplete in the time available. Thus a high urine flow rate may enhance excretion by reducing the time available for reabsorption from the tubule. Maximal acidification of the urine occurs in the most distal part of the tubule and as medullary blood flow is relatively poor, equilibration may be far from complete.

Milne continues to emphasize the significance of pH dependent excretion (Milne, 1964, 1965; Asatoor *et al.*, 1963, 1965) in pharmacological studies as well as in the treatment of poisoning. This phenomenon is important for

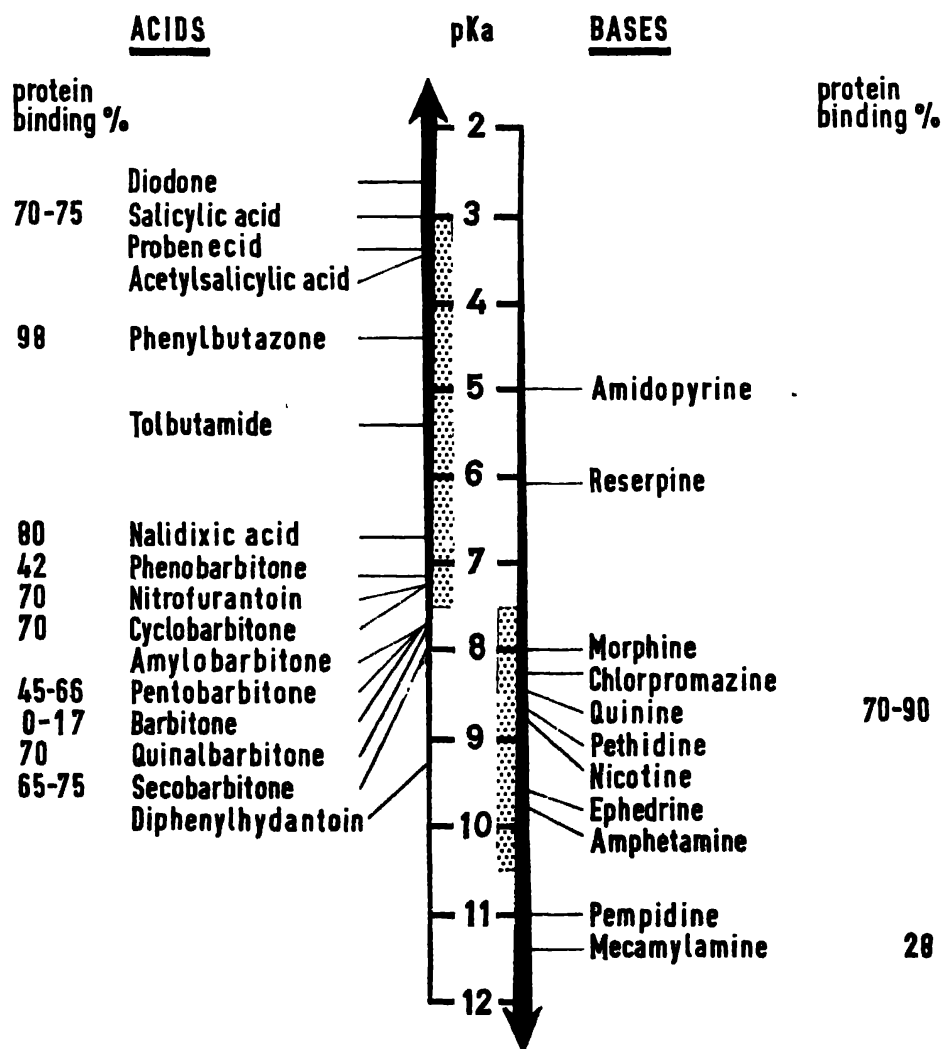


FIG. 4.4. pKa and protein binding of various drugs that are weak acids or bases. Arrows show increasing strength of acid (or base). Drugs with a pKa within the stippled range may have their excretion influenced by urinary pH change.

drugs that are weak acids with a  $pK_a$  between 3 and 7.5 and weak bases with a  $pK_a$  between 7.5 and 10.5 (see Fig. 4.4). In practice pH manipulation is of most importance in phenobarbitone ( $pK_a$  7.2) and aspirin ( $pK_a$  3.5) poisoning. These examples are discussed in greater detail.

Fig. 4.5 (MacPherson *et al.*, 1955) shows the relationship between free salicylate clearance and urinary pH. Salicylate has a  $pK_a$  of 3. The plasma levels were between 15–18 mg/100 ml with 22–44 per cent being non-protein bound. Three different agents were used to alkalinize the urine (sodium bicarbonate, acetazolamide and hyperventilation), but the effect on the free salicylate clearance was the same. This shows that urine pH and not

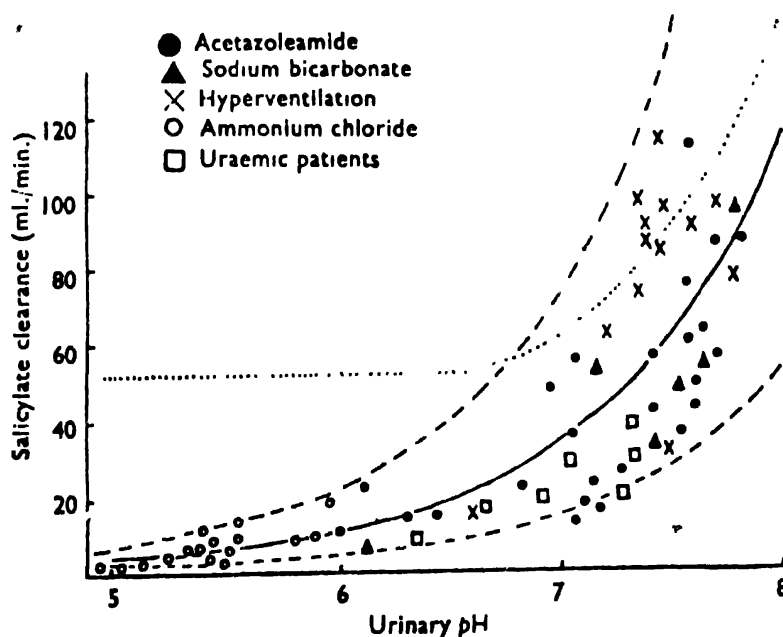


FIG. 4.5. Relation between the free salicylate clearance and the urinary pH. The calculated regression line and the limits at twice the standard deviation are shown. The upper dotted line represents the average clearance calculated from total urinary salicylate. The  $\log (C_{sa})/\text{urinary pH}$  relation remains unaffected whatever method is used to alter urinary pH. (MACPHERSON *et al.*, 1955, *Brit. J. Pharmacol.*)

the systemic acid base balance determines the clearance. These findings are best explained on the basis of non-ionic diffusion. The importance of pH manipulation in controlling salicylate excretion is obvious.

Phenobarbitone is another drug commonly used in poisoning whose excretion is affected by pH changes (Waddell & Butler, 1957). Phenobarbitone is a weak acid with a  $pK_a$  of 7.2. It is about 40 per cent protein bound. These workers found that alkalinization of the urine with intravenous sodium bicarbonate lead to an increase in the clearance of unbound drug from about 7.5 to 29 ml/min as the pH rose from 6.6 to 8 while the urine flow remained at 8–9 ml/min. The ratio urine concentration to plasma concentration rose from 0.9 to 3.6 and is of the order that could be forecast on the basis of non-ionic diffusion.

Because phenobarbitone has a  $pK_a$  close to the pH of the blood its dissociation is sensitive to minor changes in blood pH. If the intracellular pH

remains relatively constant during an acute change in the extracellular fluid and the cell membrane is permeable to the unionized but not the ionized fraction then such changes may have a profound effect on the tissue distribution of the drug. This has been well demonstrated in the dog. Severe acidosis produced by inhalation of 29 per cent  $\text{CO}_2$  lowered the blood and increased the fat and brain levels of the drug and so deepened anaesthesia: conversely, infusion of sodium bicarbonate increased the blood and lowered the brain and fat levels and so lightened coma. A similar change has been produced by hyperventilation. This effect is not due to changes in protein binding with changing pH. It underlines the importance of fully correcting any systemic acidosis and alkalinizing the urine in phenobarbitone poisoning primarily to increase excretion but also to manipulate the tissue distribution of the drug.

### **Simple Diffusion**

There is a different and less important diffusion mechanism as well as the non-ionic pH dependent one. In this second type both the ionized and unionized fractions diffuse across the tubule. This process is unaffected by pH but sensitive to changes in urine flow. When the urine volume is over 6 ml/min the flow through the nephron is so fast that the amount of drug reabsorbed is reduced. This is of most importance for relatively water soluble drugs.

The urine flow rate produced by a water load is considerably less than that readily obtainable with an osmotic diuretic such as mannitol. Even when the flow rate from the two forms of diuresis is the same the drug excretion is greater during an osmotic diuresis. A likely explanation of this is that the water from the glomerular filtrate is absorbed in different sites in the two situations. With an osmotic diuresis the tubular flow rate would be faster and so the time available for reabsorption less. Thus an osmotic diuresis has two advantages over a water diuresis in hastening drug excretion.

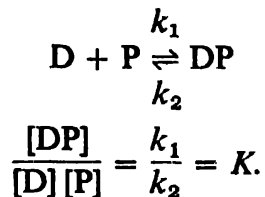
### **Protein Binding (Brodie, 1965; Thorp, 1964)**

Almost all drugs are reversibly bound to plasma or tissue proteins. If this did not happen elimination would be so rapid that the drug would have little chance of producing a therapeutic effect. The bound drug thus acts as a reservoir. Albumin appears to be the main binding protein for many drugs. This binding is reversible, remarkably non-specific and poorly understood. There are a limited number of binding sites on an albumin molecule. A drug may be in competition with natural substrates such as fatty acids, bilirubin or hormones as well as other drugs for the limited sites available. The therapeutic or toxic effect of a drug is dependent on the level of the unbound fraction. As already discussed (p. 99) it is this fraction that is available for glomerular filtration by the kidney. For many acidic drugs there is a single primary binding site. This implies a limited carrying capacity. For instance a drug with a molecular weight of 300 at a plasma concentration of over 10–20 mg/100 ml there is a rapid increase in the unbound fraction. If albumin were infused in such circumstances it would act as a reservoir and immediately reduce toxicity.

With drugs that are protein bound the percentage that is free rises as the concentration in the plasma rises. The effect of this property on their distri-

bution and elimination has been dealt with mathematically by Martin (1965a, 1965b) in some detail.

In simple terms by the law of mass action this attachment to protein may be written:



When D = drug, P = protein.

DP is drug protein complex.

$k_1$  and  $k_2$  are rates of reactions.

$K$  is the association constant.

Therefore if a harmless substance with an association constant higher than that of the toxic drug were given to a poisoned patient the unbound fraction available for glomerular filtration or dialysis would be increased. If this were done by infusion into the input of the dialyser or after production of an osmotic diuresis it could considerably enhance the removal of the toxic substance. But a rise in unbound fraction would also lead to a simultaneous rise in systemic toxicity and could be dangerous if it were not rapidly excreted. This suggestion has not yet received experimental support but could be an important therapeutic measure. The association constants of some drugs are shown in Table II.

TABLE 4.II  
ASSOCIATION CONSTANTS OF SOME DRUGS  
 $K (\times 10^{-3})$

Sulphaphenylpyrazole	460
Phenylbutazone	125
Sulphadimidine	92
Thiopental	90
Phenobarbitone	10
Salicylic acid	10
PAS	1.6

### Biotransformation

The liver is the main site of biotransformation of drugs. The enzymes are predominantly in the smooth endoplasmic reticulum of the microsomes and are protected by a lipid barrier which only fat-soluble substances can penetrate. Most reactions are oxidations to more polar metabolites. These are then readily excreted by the kidneys, unlike the more lipid soluble parent compound. Detailed information is available about the metabolism and metabolites of many drugs such as aspirin and most of the barbiturates. In contrast to this is the lack of information on others. Chlorpromazine has been on the market since the early 1950s yet when Becket and co-workers (1963) studied its metabolism in man they were able to account for only 7 per cent of a 100 mg dose. This may be because the drug is poorly absorbed, because excretion is predominantly by the bile or for other reasons. But it is a measure of our ignorance of the fate of a commonly used drug. Free chlorpromazine



and metabolites have been detected in the urine 6–12 months after cessation of therapy.

The rate at which an individual metabolizes a drug may be influenced by many factors including age, previous drug ingestion, liver disease and genetic factors. It is well known that neonates metabolize chloramphenicol (Weiss *et al.*, 1960) more slowly than older children but less well known that the half-life of salicylates varies inversely with age in children with salicylate intoxication (Done, 1962). In old-age metabolism of drugs is probably slowed though the evidence is as yet scanty.

Human liver microsomes can be induced to produce more enzyme for drug metabolism over a period of days by the administration of the same or a different drug (Burns & Conney, 1965). This may account for some of the wide variations between individuals in their ability to metabolize the same drug. Thus the half-life of a drug in one subject already on treatment with phenobarbitone may be considerably shorter than in a similar subject not previously exposed to any other drugs, or other enzyme inducers. Unfortunately, enzyme induction takes days rather than hours to appear so cannot usefully be applied to the treatment of poisoning. Other drugs may act as inhibitors and thus delay metabolism (Netter, 1962). Imipramine seriously inhibits the hepatic metabolism of phenobarbitone and meprobamate in the rat (Kato, Chiesara & Vassanelli, 1963) and may do the same in man. Chloramphenicol and iproniazid are other enzyme inhibitors. In some subjects severe liver disease may significantly lengthen the half-life of drugs (Levi, 1965). If humans behave like rats women may metabolize some drugs slower than men.

The rate at which an individual acetylates isonicotinic acid hydrazide (INAH) is genetically determined (Evans *et al.*, 1960). Approximately half the population are slow and the other half fast acetylators. But this enzyme system also acetylates phenelzine, some sulphonamides and hydrallazine (Evans & White, 1964). Therefore individuals will vary widely in their susceptibility to poisoning by these substances. (See page 213.)

### TREATMENT

The diagnosis of poisoning may be obvious or may require maximum clinical skill as well as laboratory help. The problems of diagnosis and the details of the immediate treatment required to save life will not be discussed in detail here except to re-emphasize the importance of being absolutely certain the airway is unobstructed by either bronchial secretions, vomit, false teeth or the tongue. Then an assessment is made as to the adequacy of ventilation. If the heart has stopped external cardiac massage is instituted. The state of the circulation is investigated and an attempt made to reach a diagnosis. The rectal temperature is recorded. Blood is taken for estimation of plasma levels of the poison, and pain or convulsions controlled. A decision is made about gastric lavage and consideration given to the various possible ways of promoting the removal of the poison from the body. After recovery psychiatric advice should be sought. If the current interest in dialysis and diuresis is to lead to a lowering of mortality then their use must be selective and based on some scientific understanding of the physiological principles underlying them.

**Respiratory Insufficiency (Milthers, 1963; Kirby and McNicol, 1966)**

Respiratory insufficiency is a common mode of death in poisoning.

It is a fallacy to believe that respiration may be assessed adequately by clinical examination alone. Laboratory assistance is required. Recent studies have shown that significant hypoxia is frequent in deeply unconscious patients and is present more often than could be suspected by clinical examination alone. Kirby and McNicol (1966) found an arterial  $p\text{CO}_2$  greater than 45 mm Hg in 30 per cent and an oxygen saturation below 55 per cent in 10 per cent of a group of patients with moderately severe barbiturate poisoning. Milthers (1963) reported that 3 per cent of all admissions to the Copenhagen Intoxication Centre required artificial ventilation.

Ideally facilities should be available to measure the patient's acid-base status in every hospital treating severely poisoned patients. This includes arterial pH and  $p\text{CO}_2$ . To measure oxygen saturation or  $p\text{O}_2$  is desirable but less essential. Unfortunately many centres do not have these facilities. However, the measurement of  $p\text{CO}_2$  can be made with sufficient accuracy with a simple, cheap apparatus (Howell & Campbell, 1960) which every houseman can learn to use in half an hour.

If measurements have been made and the  $p\text{CO}_2$  found to be over 45 mm Hg treatment with 100 per cent oxygen should be started. If the  $p\text{CO}_2$  is over 60 mm Hg ventilation will be required. Severe acidosis is infrequent but requires treatment when present.

Measurement of minute volume alone fails to assess the adequacy of alveolar ventilation. Still less reliance can be placed on respiratory rate. Attempts to relate alveolar ventilation to minute volume, respiratory rate, anatomical dead space and body temperature necessitate too many assumptions for the answer to be of much value.

In circumstances where treatment has to be instituted without clinical measurements then all patients with barbiturate or mixed poisonings who are unrousable need an airway inserted and the trachea cleared of secretions. Oxygen should then be given in high concentrations and be fully humidified. Patients who are cyanosed while breathing air and whose airways are clear will probably require ventilation.

Frequent physiotherapy should be given and chest X-rays taken daily while the patient is unconscious. Prophylactic antibiotics only ensure that if pneumonia does develop the organism will be resistant. It is better to prevent segmental collapse by frequent turning and physiotherapy and treat any infection appropriately if, and when, it does occur.

**Respiratory Stimulants**

Fortunately the fashion for using stimulants in barbiturate intoxication has now waned so it barely needs discussion here. Clemmesen (1963) records that when they gave up stimulation (in 1950) the mortality of barbiturate poisoning in Denmark fell from 6.3 to 3.7 per cent. "Clinically the general state of health often appears worse in a strongly stimulated patient as compared to the state of a patient who has been left alone": Myschetzky (1964) states that central stimulants are never used now in the Copenhagen Poison Centre where they have 800 admissions annually.

### Shock

Recently the mechanism of shock in barbiturate and narcotic poisoning has been investigated (Shubin & Weill, 1965). Surprisingly, peripheral vascular resistance was normal or elevated in most of the fifteen patients studied. All were in deep coma, hypotensive, often hypothermic and had respiratory difficulties, five required tracheostomy and five of the fifteen died. There appeared to be a disproportion between the blood volume and the size of the vascular bed. The cardiac output was increased by the use of vaso-pressor drugs that increased the mean arterial pressure to 90 mm Hg. Further elevation of the arterial pressure was deleterious. Large amounts of fluid, sometimes over 6 litres in 12 hours, given intravenously lead to a prompt rise in the cardiac output, blood pressure and urine flow. Generous fluid replacement is recommended for treatment of this type of neurogenic shock.

### Gastric Lavage (Harstad, Møller & Simesen, 1942)

Authorities differ on the place of gastric lavage and have argued about its value since 1929 (Sarkenstein, Rost & Pohl, 1929).

To omit gastric lavage is no longer regarded as being negligent and avoids the considerable risk of spill into the lungs. Little barbiturate is recovered unless the patient is seen early. In aspirin poisoning the subject is rarely unconscious and considerable quantities may be recovered for some hours after ingestion. At post-mortem considerable quantities of aspirin may be found in the stomach even though lavage was performed in life showing how difficult it may be to remove (Rushton, 1963). However, an attempt should be made to empty the stomach of aspirin (Beveridge *et al.*, 1964; Myschetzky, 1964). Done (1965) tends to prefer emetics to lavage unless the patient is seen within four hours of poisoning and is not unconscious.

If gastric lavage needs to be done in the unconscious patient he should be intubated with a cuffed endotracheal tube and fully oxygenated before the procedure.

### Identification of Poisons (Symposium, 1965; Curry, 1963)

Phenistix (Ames) a reagent strip was designed as a simple method for detecting phenylketonuria in infants. However, Scott (1963) found various colour changes in the urine of psychiatric patients on drugs. Phenistix go purple in the presence of aspirin or PAS, grey-purple with promazine drugs, brown with sulphonamides, green with tetracycline and grey-blue with Promethazine. Muir & Benson (1964) quantitated the aspirin responses and found the paper reacted with the non-protein bound fraction only. The paper was dipped into separated serum and read immediately. No colour was produced until a level of 20 mg/100 ml was reached. At 20–45 mg/100 ml they became faint mauve and above 50 mg/100 ml strong mauve: in urine or other aqueous solution levels of 2.5 mg/100 ml were detected. These sticks can therefore be a useful and rapid screening test for the presence and concentration of salicylate in blood and urine. The presence of salicylate in urine may also be detected with ferric chloride. The estimation of the serum salicylate level by Trinder's (1954) method takes about 5 minutes.

To know the concentration of barbiturate in the serum of a poisoned

patient without any knowledge of which one has been taken is of limited value to the clinician. Because of their different protein binding, lipid solubility, mode of excretion and pKa it is necessary to know at least whether the drug is long acting, e.g. phenobarbitone (relatively lightly protein bound and largely excreted unchanged by the kidneys) or intermediate, e.g. pentobarbitone (highly protein bound and largely metabolized by the liver). Above 10 mg/100 ml is the dangerous level for a long acting barbiturate and over 3 mg/100 ml for an intermediate one. Until the advent of thin layer chromatography the partial identification of a barbiturate was relatively complex and time consuming. With the new technique the partial identification can be rapidly achieved (Podmore, 1962; Cochin & Daly, 1963). A guide to the serum level may be found by the 5 minute estimation of Wallerius, Zaar & Lansing (1963) or Curry's (1964) modification though most workers usually still confirm the levels by Broughton's (1956) technique.

The urgent identification and quantitation of other poisons is beyond the scope of most routine laboratories. But this should not deter the physician from taking and preserving suitable samples of blood and urine for analysis in specialist laboratories if necessary.

**Forced Diuresis** (Myschetzky & Lassen, 1963; Ohlsson & Fristedt, 1962; Beveridge *et al.*, 1964; Ghose & Joekes, 1964; Linton *et al.*, 1964; Cumming, Dukes & Widdowson, 1964)

Forced diuresis is now an established technique. It is safe if carefully used but dangerous and possibly lethal if unsupervised. The technique will therefore be described in some detail. The relative contraindications include severe heart or renal disease.

The basic principles are:

- |  |   |  |
|--|---|--|
| <ol style="list-style-type: none"> <li>1. Establish closed urinary drainage with an indwelling catheter.</li> <li>2. Set up I.V. infusion with a large bore cannula, correct fluid deficit and provide a fluid load to encourage urine flow.</li> <li>3. Administer an osmotic diuretic with or without a pharmacological diuretic.</li> <li>4. Continue I.V. therapy with a flow rate of 500 ml/hour.</li> <li>5. At 3-4 hours assess urine output:               <ul style="list-style-type: none"> <li>If now 6 ml/min or more continue treatment.</li> <li>If less than 1 ml/min stop treatment.</li> <li>If between 1 and 6 ml/min proceed with caution.</li> </ul> </li> <li>6. Maintain mild osmotic diuresis.</li> <li>7. Give adequate potassium.</li> <li>8. Keep careful 8-hourly balance charts.</li> <li>9. Watch clinical state very closely.</li> </ol> | } | <i>Within<br/>First<br/>2 to 3<br/>Hours</i> |
|--|---|--|

In the first 2-3 hours 1.5-4 litres of fluid are required for the average adult to correct dehydration, combat shock and promote urine flow. Because of the danger of giving a powerful osmotic diuretic before correcting dehydration, mannitol is often not given until the second hour. 200-300 ml 20 per cent mannitol can then be infused over the next hour into a side arm of the drip or into a second drip and then slowed to 100 ml in 4-6 hours to maintain the advantages of a solute load. Intravenous ethacrynic acid or frusemide have a very powerful and rapid diuretic action and are used to initiate a

vigorous diuresis by some workers. Alkalinization is usually achieved by giving 500 ml 2 or 3 per cent  $\text{NaHCO}_3$  or M/6 sodium lactate. A commonly used fluid rota is 500 ml alkali, 500 ml 5 per cent glucose and 500 ml isotonic saline in turn omitting the bicarbonate if the urine pH reaches 8. If a pH meter is not available to monitor the urine, narrow range indicator papers may be used. Litmus paper is useless.

This regime contains very large quantities of sodium but appears to be safe if diuretics are used with it. If there is concern about the sodium intake this may be avoided by giving 5 per cent glucose as required. A urine flow rate of 500 ml/hour is readily achieved (12 litres/24 hours) in most patients and 15–20 litres/24 hours may be reached if needed.

Three to four hours after initiation of therapy its success must be estimated. If the urine flow is below 1 ml/min there is renal failure and therapy should be stopped and the patient considered for hæmodialysis. If the flow rate is between 1–6 ml/min the diuresis is poor and the rate of IV infusion must be severely reduced. A flow rate of over 6 ml/min is satisfactory and reached in the majority of cases. When a satisfactory diuresis has been initiated at least 25 mEq (2 g) every 6 hours of potassium chloride should be given. In salicylate poisoning considerably more may be required and the serum level should be checked every 8 hours.

The increased excretion of drug during an osmotic diuresis compared to an equivalent water diuresis is the reason for continuing mannitol.

Careful 8-hourly fluid balances and meticulous watch on the clinical state are essential. Pulmonary œdema is an obvious risk but has only been reported rarely (Linton, *et al.*, 1964).

Ohlsson & Fristedt (1962) used this form of therapy over a 10-year period (1950–1959) routinely in 605 cases. The average urine output in severe cases was 7 litres. There were no patients who developed pulmonary œdema.

Repeated careful clinical examination may mean the patient remains uncovered and exposed for long periods of time. Heat loss may be considerable and a check must be kept on the rectal temperature to prevent hypothermia.

The margin of safety for infusion of large quantities of sodium and alkali is wide while there is a good urine output and there is no potassium deficiency, as the normal kidney has a large reserve capacity to excrete bicarbonate. The danger of metabolic alkalosis is minimal unless hypokalaemia is impairing the kidneys' ability to excrete bicarbonate.

The contrary, however, is not true. If an acid urine is required to promote the excretion of weak bases such as pethidine or pempidine the margin is small. Obviously the alkali is omitted from the rota but rapid acidification is more difficult to achieve and is perhaps best done with arginine hydrochloride or lysine hydrochloride 10 g being given intravenously over 30 minutes. The kidneys may take several hours to achieve maximal acidification. This may be followed with oral ammonium chloride 4 g 2 hourly for 3 doses.

### Dialysis

The clinical dialysis of poisons has been reviewed in detail by Maher & Schreiner (1965). The criteria for assessing the place of dialysis in the treatment of poisoning have been stated by Schreiner (1958).

1. The poison molecule should diffuse through a dialysis membrane,

such as cellophane, from plasma water and have a reasonable removal rate or dialysance.

2. The poison must be sufficiently well distributed in accessible body fluid compartments. If substantial fractions of the absorbed poison are bound to protein, concentrated in inaccessible fluid compartments (e.g. cerebrospinal fluid) or attain a significant intracellular concentration, then effective dialysis will be limited. This restriction is diminished, however, if the sequestered portion rapidly equilibrates with the plasma.

3. There should be a relationship between the blood concentration, the duration of the body's exposure to the circulating poison and toxicity.

4. The amount of poison dialysed must constitute a significant addition to the normal body mechanisms for dealing with the particular poison under consideration. This includes metabolism, conjugation and elimination of the substance by bowel and kidney.

The discerning application of these principles has been hampered by the lack of adequate evidence on which to base a judgement.

In order to assess the place of these vigorous, often expensive methods of treatment that are not without risk it is necessary to know numerous facts which are not always available. These include:

1. The morbidity and mortality of previous methods of treatment.
2. The mean and if possible some idea of the variation in the plasma half-life in different individuals of the various drugs at different plasma levels. The  $t_{\frac{1}{2}}$  during treatment may then be compared to this.
3. The renal clearance and half-life of unchanged drug at various rates of urine flow and at different pH.
4. The clearance and half-life of unchanged drug during hæmodialysis and peritoneal dialysis.

Many published papers record in great detail the amount of drug collected in the urine or dialysis fluid. As the methods of estimation of a drug often also estimate its more water soluble metabolites which have a higher clearance—these figures are difficult to interpret. Except in the presence of oliguria metabolites only form a small percentage of the total blood level. This data would be more valuable if the change in blood levels with time were also reported.

In the hours after a dialysis the blood level of drug usually rises as it diffuses out of body fat and other stores. This rebound effect has long been recognized for urea in renal failure but also occurs with drugs. To obtain a clear picture of the value of the procedure blood levels need to be measured at completion of dialysis and again 8–12 hours later. In barbiturate poisoning particularly the blood level of the drug may rise relatively late in the illness. This is attributed to continued absorption from the gut during reactivation of a narcotized gastrointestinal tract. Thus in cases for publication the half-life should be calculated from plasma levels measured at least every 24 hours.

The duration of coma provides a very blurred end-point by which to compare the effects of various treatments. Though where the effect is striking—as with the very long acting barbiturates in some of the Scandinavian reports, this can be useful.

In more borderline situations, as with the short or medium acting barbiturates and the newer psychotherapeutic drugs the place of diuresis or

dialysis is far from clear. Information is urgently needed to make a judgement on the place of these methods in treatment.

## INDIVIDUAL POISONS

### Salicylates

Salicylates have profound effects upon metabolic processes and the acid-base balance of the body. The situation is complicated as these effects vary with age and are most severe in infants. At all ages salicylate intoxication produces hyperventilation leading to a fall in  $p\text{CO}_2$  and rise in pH. This tends to be the main abnormality in children aged over 4 years and adults who are moderately intoxicated. In infants and children under 4 years and in very severe intoxication in adults, metabolic acidosis may be the dominant disturbance. There appears to be an accumulation of organic acids mainly aceto-acetate and hydroxybutyrate secondary to disturbances of intermediary metabolism. There is therefore a mixed disturbance. In children under 3 years of age the blood pH is usually low while in adults it is high. The urine pH is not a good guide to the blood pH in this situation and is often confusing. The clinical picture alone, unless there are convulsions, hyperpyrexia, coma, severe dehydration or pulmonary oedema may be a poor guide to the severity of the intoxication.

The serum salicylate level must be interpreted in relationship to the time after ingestion and whether a single or multiple doses have been taken.

Done (1960) has suggested that the extrapolated zero time level  $S_0$  is the best biochemical guide to severity and this has been confirmed by others (Cumming, Dukes & Widdowson, 1964). This is based on the fact that the disappearance of salicylate from the blood after a single dose approximates to a first order reaction. When a single dose has been taken for more than 6 hours before the blood level was measured an extrapolated zero time level may be calculated. For this calculation the average disappearance rate is taken. In Done's series the mean  $t_{1/2}$  of 17 patients was 20 hours (range 15–29 hours). In other series the mean is 22 hours with range of 6–40 hours.

$$\text{Then } \log S_0 = \log S - bT$$

$$\therefore \log S_0 = \log S - 0.015T$$

Where S = salicylate level measured

$S_0$  = extrapolated zero time level

b = slope for average  $t_{1/2}$

T = time in hours after ingestion.

Alternatively the  $S_0$  value can be read off from Done's published nomogram (see Fig. 6). He found:

$S_0$ = less than 50 mg/100 ml	not intoxicated
50– 80 mg/100 ml	mild intoxication
80–100 mg/100 ml	moderate intoxication
110–160 mg/100 ml	severe intoxication
over 160 mg/100 ml	not compatible with life.

The salicylate level after a single dose continues to rise for about 6 hours due to continued absorption. If the time between blood sample and drug

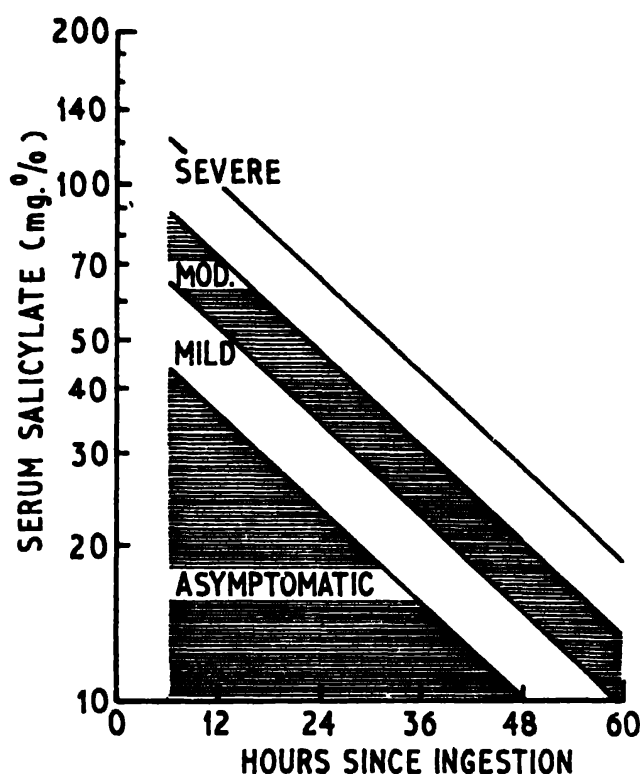


FIG. 4.6. Nomogram relating serum salicylate concentration and expected severity of intoxication at varying intervals following ingestion of a single dose of salicylate. (After DONE, A. K., 1960, *Pædiatrics.*).

ingestion is less than 6 hours a correction may be applied assuming that absorption doubles the serum level in 4 hours. This calculation continues to give the best biochemical estimate of severity.

Attention was drawn to the increase in salicylate excretion by alkali therapy in 1931 (Morris & Graham, 1931). Yet it is still not universally used in salicylate poisoning. The dangers of alkali have probably been over emphasized and have prevented many patients receiving the benefit of this treatment. The problems usually arise in this way. A low bicarbonate with hyperventilation is interpreted (incorrectly) as signifying a metabolic acidosis and no blood pH measurement is made. Alkali is given but the urine pH fails to rise. More alkali is prescribed and the potassium deficit remains uncorrected. Tetany or respiratory difficulties may then occur.

Considerable potassium deficiency may be present and the deleterious effects of this are increased by vigorous sodium bicarbonate therapy. When blood pH is high the renal tubules conserve hydrogen ions by potassium exchange thus aggravating potassium depletion. There is an increased reabsorption of bicarbonate in the presence of hypokalæmia which may aggravate the alkalosis already present and hinder alkalization of the urine. In this situation potassium chloride needs to be given in large doses. When a good urine flow has been established there is little danger from generous potassium replacement. Robin, Davis & Rees (1959) gave 240–340 m-mole KCl in 24 hours to adults with considerable improvement in their clinical state.

Treatment by alkalization and forced diuresis is usually safe even if the



TABLE 4.III  
PLASMA HALF-LIFE ( $t_{1/2}$ ) AND CLEARANCE OF SOME DRUGS UNDER DIFFERENT CONDITIONS. DATA FROM VARIOUS AUTHORS.

<i>Drug</i>	<i>t<sub>1/2</sub> Hours</i>			<i>Clearance ml./min</i>		
	<i>Untreated</i>	<i>Forced Diuresis</i>	<i>Hemo-dialysis</i>	<i>Untreated</i>	<i>Forced Diuresis</i>	<i>Hemo-dialysis</i>
Aspirin	20	3-7.5	3.5-6		95	
Phenobarbitone	74-94	?-48	4-9		8-29	40-70
Medium-acting Barbiturates	11-37	?-15	3-17		5-20	25-40
Bromide	332	16-37	0.9			

initial arterial pH is raised (Dukes *et al.*, 1963). Infusion of alkali in their patients was uniformly accompanied by a rise in blood pH (mean 7.51, highest 7.69). The  $p\text{CO}_2$  rose only slowly. They recorded no harmful effects from the deliberate production of a metabolic alkalosis and found the enhanced excretion of salicylate of great benefit.

Other disturbances that may occur include dehydration, hyperglycæmia, hypoglycæmia, hyper- or hyponatræmia, a bleeding tendency (usually due to hypoprothrombinæmia and correctable by Vitamin  $\text{K}_1$  but sometimes due to thrombocytopenia), proteinuria with casts and rarely renal failure. Pulmonary œdema is partly a hypersensitivity phenomenon. Coma and hyperpyrexia are very dangerous signs. Hæmorrhagic gastritis may be the cause of a falling blood pressure.

### Treatment

Mild poisoning can well be managed by giving fluids and electrolytes by mouth. Vitamin  $\text{K}_1$  should be given routinely. All other cases need I.V. replacement. Moderate and severe cases need a careful but vigorous programme of forced alkaline diuresis with generous potassium replacement. This should be started on admission before the patient reaches the ward.

Table 4.III shows in summary the  $t_{\frac{1}{2}}$  of salicylate calculated from various published figures. Both alkaline diuresis and hæmodialysis reduced the  $t_{\frac{1}{2}}$  to one third or one quarter of the untreated value. The slightly faster  $t_{\frac{1}{2}}$  with hæmodialysis is of no clinical significance in itself. The delay in initiating hæmodialysis offsets this advantage. Hæmodialysis is normally only performed for 6–10 hours though it can readily be repeated. Alkaline diuresis has no time limitations and is practicable in any hospital. The indications for hæmodialysis are mainly clinical. Renal failure and failure to produce a urine flow rate of over 1 ml/min after adequate fluid and diuretic therapy is a strong indication for dialysis: it should also be considered when there is clinical deterioration despite an adequate diuresis. In comparing the efficiency of various methods of treatment false conclusions are reached when the wrong comparisons are made. To show that the  $t_{\frac{1}{2}}$  during no treatment is longer than the  $t_{\frac{1}{2}}$  during dialysis does no more than show that dialysis is an effective way of removing salicylate—which is already known.

### BARBITURATES

Barbiturates are usefully subdivided into three groups—long, medium and ultrashort acting (Table 4.IV). The subdivisions of the medium into intermediate and short is not useful though it must be realized that there is considerable variation in this group.

Renal excretion is by glomerular filtration and diffusion in the tubules (see p. 98). Active proximal tubular secretion does not occur.

Poisoning with barbiturates is commonly accompanied by a number of complications which need assessment and may need treatment. Respiratory depression is the most important and dangerous (see p. 107). The maintenance of a clear airway and the scientific measurement of respiratory insufficiency are vital actions to be taken if mortality is to be reduced.

Hypothermia is particularly common after poisoning with one of the medium acting groups of barbiturates. This leads to a fall in the basal meta-

TABLE 4.IV  
PROPERTIES OF BARBITURATES

<i>Duration of Action</i>	<i>Example</i>	<i>Protein Binding</i>	<i>Lipid Solubility: Partition Coefficient Methylene Chloride and Water</i>	<i>Per cent Metabolized</i>
Long	Pheno-barbitone	40%	3	10%
Medium	Pento-barbitone	55-75%	40	80%
Ultrashort	Thiopentone	75% +	500 +	90% +

bolic rate and as metabolism is the major method of elimination in this group—to a serious prolongation in the half-life. The response to treatment by rapid rewarming and intravenous hydrocortisone is good. Artificial ventilation and forced diuresis corrects the metabolic acidosis, often found in hypothermia. When patients with barbiturate poisoning are hypothermic they may have polyuria and a high barbiturate clearance (Linton & Ledingham, 1966). This may be due to lack of production of ADH or insensitivity of the tubules to this hormone.

Uncomplicated barbiturate intoxication leads to oliguria by overproduction of ADH (De Bodo & Prescott, 1945) and by a reduction in the glomerular filtration rate. To produce a diuresis it is important to give an osmotic diuretic.

Another complication of barbiturate poisoning is ischaemic contractions of muscle due to a combination of hypoxia and pressure. Rather characteristic bullous lesions of the skin have been described in 6.5 per cent of 290 patients with barbiturate poisoning (Beveridge & Lawson, 1965). They were not seen in other poisonings and therefore thought to be of diagnostic significance. However, Sørensen (1963) has described similar lesions in a variety of poisonings including carbon monoxide, Tofranil, acetyl carbromal, glutethamide, methadone alcohol, meprobamate, dihydrocodein and alcohol. It seems difficult therefore to regard the lesions as diagnostic of barbiturate ingestion.

The majority of patients with barbiturate overdose are mildly intoxicated and only require conservative therapy (Matthew & Lawson 1966). About 10% will require really vigorous treatment if the present mortality rate of 10% in this severe group is to be lowered. Lee and Ames (1965) have had remarkable successes even in apparently hopeless situations.

It has been suggested that if all patients who were very drowsy or unconscious from barbiturate poisoning were started on a regime of forced diuresis in the casualty department the need for haemodialysis, the morbidity and the mortality would considerably diminish (Lee & Ames, 1965). The medical and nursing staff rapidly learn to apply the technique with little risk to the patient. When the results of the blood barbiturate analysis become

available the decision can be made as to how vigorously the therapy should be continued. An osmotic purge such as sorbitol should be used to empty the gut of unabsorbed poison. Magnesium salts should be avoided as some may be absorbed and exacerbate respiratory depression.

Hæmodialysis with a Kolf twincoil or a Kiil kidney is the most effective method of rapidly lowering a very high blood level whichever barbiturate is involved. Peritoneal or minicoil dialysis as ordinarily employed have very little place in such circumstances.

If forced diuresis has been adequately initiated, is proceeding well and the clinical state is not deteriorating it should be continued when high blood levels are reported unless facilities for hæmodialysis are immediately available. The time lost in transferring the patient to a dialysis centre probably outweighs the advantages of the procedure. But if the clinical state is deteriorating, the blood level is very high (over 15–20 mg/100 ml for phenobarbitone or over about 6–8 mg/100 ml for intermediate barbiturates) and particularly if the blood pressure progressively falls, then hæmodialysis should be instituted as soon as practicable. Peritoneal dialysis may have a limited place especially where hæmodialysis is not readily available. A blood barbiturate of more than about 10 mg/100 ml for phenobarbitone or other long acting barbiturates and 3–4 mg/100 ml for short and medium acting barbiturates should be regarded as severe. If the barbiturate is only one of several drugs ingested then a considerably lower level may be dangerous.

Table 4.III shows in summary what may be expected from various forms of treatment. In phenobarbitone intoxication as already indicated it is important to fully alkalinize the urine. In “untreated” intoxications the  $t_{\frac{1}{2}}$  is 74–94 hours whereas during hæmodialysis it may fall to between 4.5–10 hours. For alkaline diuresis it has been recorded at 48 hours at a clearance of 8 ml./min but considerably higher clearances are obtainable so the half-life should probably be from 20–48 hours. Adequate figures are not available for peritoneal dialysis.

Medium acting barbiturates may be metabolized at up to about 4 per cent per hour giving a  $t_{\frac{1}{2}}$  of around 17 hours. Metabolism is normally the major method of inactivation (Brodie *et al.*, 1953). In general hæmodialysis will lower a high barbiturate level faster than any other method. It is least efficient for highly protein bound and lipoid soluble drugs such as secobarbitol when the clearance is low. Peritoneal dialysis is only slightly more efficient than forced diuresis. Alkalinization of the urine has no effect on tubular reabsorption as the pKa of all the members of this group is 7.8 or over. In general the clearance is directly related to the urine flow. But mannitol and other osmotic diuretics, uniformly increase the clearance above that produced by water (Cirksena *et al.*, 1964). Twenty-four hours of forced osmotic diuresis is usually at least as beneficial as 8 hours hæmodialysis. Detailed figures are not given for the various intermediate acting barbiturates as they are not all available.

The number of careful critical studies published is extremely small (Bunn & Lubash, 1965) and many more need to be done. But a single case may be valuable as shown by Bloomer (1965). He found that though alkaline diuresis produced considerable increases in the clearance of pentobarbitone this only amounted to about 20 per cent of hepatic transformation. Peritoneal

dialysis was not more effective. He felt their place in the treatment was limited.

In summary the present situation in barbiturate poisoning is:

1. Forced osmotic diuresis may be required in less than 10 per cent of patients, but in these it may be life saving. It is of greatest importance with long acting barbiturates.

2. Early initiation of forced osmotic diuresis may prevent the need for dialysis. It will maintain the circulation and prevent metabolism being reduced. It will alert and train nurses, doctors and laboratory staff in the careful management these patients may require. Severe poisoning will be recognized earlier and their treatment applying these techniques will not be a unique and risky experience for all concerned. Forced osmotic diuresis should be started in all deeply unconscious patients with barbiturate poisoning. Alkalinization of the urine is most valuable when phenobarbitone is the barbiturate.

3. Hæmodialysis is necessary:

- (a) When renal failure is present.
- (b) When the clinical state deteriorates during diuresis with a falling blood pressure or deepening coma.
- (c) If the blood barbiturate level continues to rise.
- (d) In very severe poisoning.

4. Peritoneal dialysis may be useful if hæmodialysis is not immediately available. Newer techniques with peritoneal dialysis may considerably enhance its application in the future.

5. While thinking about these methods of reducing the blood barbiturate level do not forget the airway and respiratory insufficiency.

### **Amphetamine**

The plasma level of amphetamine is very low even after large doses indicating extensive extravascular binding. But amphetamine is a weak base with a pKa 9.8 (Fig. 4.4) and urinary excretion data conclusively show the effect of pH change (Beckett & Rowland, 1965; Asatoor *et al.*, 1965). The biological half-life is about 5 hours in acid urine and many times longer in alkaline urine. Forced diuresis with acidification should be employed routinely in amphetamine intoxication.

### **Pethidine**

Pethidine is a weak base with a pKa 8.6 (Fig. 4.4). Its renal excretion is pH dependent (Asatoor *et al.*, 1963). In maximally acid urine pH 4.8–5 the  $t_{\frac{1}{2}}$  after a therapeutic dose is approximately 16 hours. The  $t_{\frac{1}{2}}$  due to metabolism is about 4 hours. In pethidine poisoning and after accidental ingestion in the presence of an amine oxidase inhibitor acidification of the urine would considerably enhance the excretion of the drug.

### **Bromide**

Bromide poisoning is now unusual in England and it is rare for it to be lethal. However, recovery may be slow. The  $t_{\frac{1}{2}}$  may be reduced from 65 hours when on salt treatment to 37 hours with an osmotic diuresis, 16 hours with a

diuretic and osmotic diuresis and about 1 hour with hæmodialysis (Wieth & Funder, 1963).

#### **Methanol (Jørgensen & Wieth, 1963)**

This is highly toxic and slowly metabolized. Early correction of the metabolic acidosis is important. Clearance is rapid with hæmodialysis which is the treatment of choice if it can be instituted without delay. The  $t_{\frac{1}{2}}$  is reduced about 40 times to 1·2 hours. If there has to be delay before hæmodialysis can be started peritoneal dialysis is a fair substitute. Ethanol given to prevent the production of toxic metabolites has only a transient effect. Fructose should not be used as it enhances their production.

#### **Iron (Gervirtz & Rausen, 1966)**

Many iron tablets still look like a popular brand of children's sweets. It has been suggested that all iron preparations supplied to women, and in particular to pregnant women (as they are liable to have other small children), should be the unattractive preparations such as ferrous fumarate (Glaxo). Children aged 1–2 are most commonly poisoned and death has occurred after as little as 3 g. Vomiting should be induced immediately and then 5–10 g desferrioxamine B given by Ryles tube. This should be followed by an osmotic purge. Up to 2 g of chelating agent may be given slowly intravenously. Adequate intravenous fluids are prescribed and the acidosis corrected. The correct dose of desferrioxamine to use both intragastrically and intravenously is still debated but should not prevent the prompt use of this chelating agent as it is an important advance in the treatment of iron poisoning.

#### **Imipramine, Amytriptyline**

In spite of albumin binding plasma levels of these drugs tend to be low because of tissue protein binding. Urinary excretion of unchanged drug is low (about 1 per cent) but no studies with pH manipulation have been published. Any increase in acid urine is likely to be unimportant. Metabolism is the main method of removal. Clinical improvement during dialysis and forced diuresis when insignificant amounts of unchanged imipramine were removed raises the possibility that some metabolites are toxic (Prout, Young & Goddard, 1965).

The clinical picture is variable. Coma is usually deep and respiration slow. Respiratory arrest may occur suddenly and unexpectedly. Both hyper- and hypotension have been reported. Cardiac abnormalities including ventricular flutter or tachycardia and atrial fibrillation and tachycardia. The mechanism may be a direct cardiac toxicity or vagally mediated. Temperature regulation is frequently disturbed. The pupils may be normal or dilated and unresponsive to light.

Recovery has followed very severe poisoning (e.g. after 5,375 mg imipramine) even when fits and cardiac arrest complicated the course. Forced diuresis is of possible but unproven value.

#### **Glutethimide (Doriden)**

Poisoning with glutethimide is often severe. It is highly lipid soluble and is rapidly distributed into the liver and body fat after ingestion. Plasma

levels are low and almost no unchanged drug appears in the urine. Its removal is not greatly enhanced by diuresis or dialysis. There is a tendency to pulmonary oedema that may be aggravated by forced diuresis. Shinaberger and coworkers (1965) have shown a considerably increased clearance by using lipid dialysis thus capitalizing on the high lipid solubility of the drug. This technique has yet to be used in man. Ingestion of more than 10 g of glutethimide or a blood level above 3 mg/100 ml. has a poor prognosis and haemodialysis should be considered and may have to be repeated.

#### **Carbon Monoxide (Douglas *et al.*, 1962; Ledingham, 1964)**

The most important feature in the treatment of carbon monoxide poisoning is to promote the rapid elimination of this gas from the blood. The development of pressure chambers has presented the opportunity to study the effect of hyperbaric oxygen on recovery from carbon monoxide poisoning. Initial studies were on dogs gassed to produce a blood level of carboxyhaemoglobin of 70 per cent. The time taken to reduce this to 35 per cent (i.e. the  $t_{1/2}$ ) was measured during treatment with oxygen at 2 atmospheres and during conventional therapy. The hyperbaric oxygen took less than half the time of other treatments. Oxygen corrects cellular anoxia rapidly as it dissolves in the plasma as well as expelling carbon monoxide. All the patients treated recovered consciousness in 30–90 minutes and there were no deaths and no irreversible neurological or myocardial damage. Abnormal ECG rapidly reverted to normal. In order to be effective treatment has to be instituted early. This method of treatment is clearly valuable and may be life saving for those centres able to afford it.

These examples have been chosen as they are common agents in poisoning and because modern therapy has a rational basis. A major compendium on industrial poisoning has been published (Browning, 1965) and the poison information centres are able to give detailed advice on less common agents.

### **RECENTLY SUGGESTED TREATMENTS**

#### **Ion Exchange Resins**

Edwards (1964) has suggested that ion exchange resins may be useful in the early treatment of poisoning particularly with highly protein bound, poorly dialysable drugs which are largely metabolized by the liver. He protected rats poisoned with secobarbital by feeding them with cholestyramine. This anion exchange resin, particularly if combined with an osmotic purge, might well prevent further absorption and hasten excretion of such a drug. Unfortunately, exceedingly little has been discovered about the enterohepatic circulation of drugs so whether this measure would assist excretion after absorption is unknown.

However, Williams, Smith & Millburn (1965a,b) have systematically studied the biliary excretion of a number of compounds in the rat. The results suggest that any large foreign organic compound containing two or more aromatic rings or their equivalent is likely to be excreted in the bile in appreciable amounts especially if it can be converted into a conjugate such as a glucuronide. If it is already a conjugate or has a structure similar to a conjugate and is of a certain size (i.e. molecular weight 300–400) then it

likely to be excreted in the bile in significant amounts. It could thus become available for enterohepatic circulation.

### Charcoal

Yatzidis and coworkers (1965) have described a technique of hæmo-perfusion through charcoal. They claim it is cheap, easy to do, and quick, requiring little preparation and only sterilization of the micro apparatus. They reported remarkable clearance of phenobarbitone and barbitone in two cases of poisoning. This has been further tested by Dunea & Kolff (1965). They found it safe with few side effects and felt it deserved further study.

### Lipid Dialysis

Because of the difficulties in trapping highly lipid soluble drugs in aqueous perfusion fluid in hæmo- or peritoneal dialysis, Shinaberger and coworkers (1965) have used lipid dialysates. In this technique the usual isotonic water and salt solution is replaced by lipid. They dialysed dogs poisoned with glutethimide and found considerably enhanced removal. This method may well have an application in man poisoned with very highly lipid soluble drugs (such as glutethimide) as these are poorly removed by hæmodialysis or forced diuresis.

### Tham (Setter *et al.*, 1964)

Tris (hydroxymethyl) aminomethane (Tham) is a buffer that has been used both intravenously to promote the excretion of weak acids and intra-peritoneally to enhance this clearance during peritoneal dialysis. Though it may maintain the pH in the peritoneum above 8 and so concentrate intermediate acting barbiturates (with pKa's over 7.8) its place in treatment has not yet been well defined.

## EMERGENCY TREATMENT OF POISONING

### All Poisonings are Emergencies

1. Ensure the air passages are clear (tongue, teeth, food, vomit, bronchial secretions). Insert an airway if the patient is unconscious.
2. Remove from the source of poison (e.g. coal gas).
3. Confirm the heart is beating. If not ventilate and massage (external).
4. Assess ventilation by clinical measurement and not clinical judgement. If inadequate treat. If unconscious give patient 100 per cent O<sub>2</sub>.
5. Assess circulatory state—B.P., pulse, hydration. Treat.
6. Take blood samples for acid-base status, drug identification and quantitation.
7. Rectal temperature. Remember low reading thermometer.
8. Is there a specific antidote in this case? (Unlikely.)
9. Control convulsions and pain.
10. Think about gastric lavage and emesis. Dangerous if unconscious. Useless (except for aspirin) if more than 4 hours since ingestion.
11. Give adequate fluids. Treat acid-base disturbances.



12. Promote excretion:
  - (a) Will forced diuresis help?
 

Salicylates and phenobarbitone, yes.  
Intermediate barbiturates, probably.  
Unknown polypharmacy, possibly.  
If in doubt and the patient is unresponsive, start diuresis then ask advice.
  - (b) Should urine pH be controlled? (See Fig. 4.4.)
  - (c) Will dialysis be necessary?
 

Only in the severest 2–5 per cent and the neglected patients.
13. Forget about respiratory “stimulants” and “prophylactic” penicillin.
14. Turn 2-hourly. Physiotherapy. X-ray chest.
15. Saving severely poisoned patients is difficult as well as hard work. Call for help. On occasions anaesthetists, biochemists, cardiologists, pharmacologists, pathologists, bronchoscopists and surgeons are needed. Call them. Remember the Poisons Information Centres.
16. Call the psychiatrist.

### POISONS CENTRES

LONDON	Poisons Reference Service New Cross Hospital Avonley Road London S.E.14.	Tel.: 01-407 7600
EDINBURGH	Scottish Poisons Information Bureau The Royal Infirmary Edinburgh 3.	Tel.: 031 FOU 2477
CARDIFF	Poisons Information Centre The Cardiff Royal Infirmary Cardiff.	Tel.: 0222 33101
BELFAST	Poisons Information Centre Royal Victoria Hospital Belfast.	Tel.: 0232 30503
DUBLIN	Poisons Information Centre Jervis Street Hospital Dublin 1.	Tel.: Dublin 45588
LEEDS	The Casualty Department The General Infirmary Leeds 1.	Tel.: 0532 32799

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CHAPTER 5

DISORDERS OF THE ADRENAL CORTEX  
AND PITUITARY GLAND

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THE adrenal gland is, in effect, two separate endocrine glands which differ in development, structure, and function. The adrenal medulla is part of the sympathetic nervous system and is relatively unimportant. The adrenal cortex, on the other hand, plays a vital role in health and disease. This chapter is concerned with the physiology of the adrenal cortex and its inter-relationships with the pituitary gland, with the measurement of adrenocortical activity in man, and with the assessment of pituitary-adrenal function in some of the disorders which affect these glands.

**PHYSIOLOGY AND BIOCHEMISTRY OF THE ADRENAL CORTEX**

All the known hormones of the adrenal cortex belong to the group of substances known as steroids. Steroids have a common molecular structure made up of 17 carbon atoms, known as the cyclopentenophenanthrene nucleus, and differ only in the nature and position of the radicals attached to the various carbon atoms. Many of the steroids formed by the adrenal cortex have 4 additional carbon atoms attached to this cyclopentenophenanthrene nucleus, contain 21 carbon atoms in all, and are therefore known as  $C_{(21)}$  steroids. The main raw material for the biosynthesis of adrenal steroids is cholesterol, itself a steroid, but other routes of synthesis have been described in which acetate is the starting point. Over 40 different steroids have been isolated from extracts of the adrenal glands of animals, but most of them are either precursors or metabolites of the active hormones (Cope, 1965).

It is customary to divide the hormones of the adrenal cortex into three main groups according to their physiological activity.

1. *Glucocorticoids* are  $C_{(21)}$  steroids which have an effect on carbohydrate metabolism, their action being opposed to that of insulin. In excess, these substances stimulate gluconeogenesis and produce a negative nitrogen balance which is accompanied by retardation of growth, muscle wasting, thinning of the skin and osteoporosis. Glucocorticoids, and their synthetic analogues, also have an anti-inflammatory action which has been widely used in the treatment of non-endocrine disorders.

2. *Mineralocorticoids* are  $C_{(21)}$  steroids which promote the retention of sodium and excretion of potassium by the kidneys. The properties of these two groups of steroids are not mutually exclusive and some glucocorticoids also have a mineralocorticoid effect.

3. *Androgens* are  $C_{(19)}$  steroids. Their masculinizing activity is weak compared with the principal male hormone, testosterone, secreted by the testes. Their role in the body is still uncertain although it is generally believed that they have anabolic properties and thus promote nitrogen retention.

Progesterone and 17  $\alpha$ -hydroxyprogesterone have been detected in very small amounts in the adrenal venous blood of women following treatment with ACTH to cause maximal adrenal stimulation (Short, 1960). The significance of these observations remains uncertain for both these steroids are formed during the biosynthesis of glucocorticoids and mineralocorticoids and these trace amounts may merely represent a "leakage" of precursors from the adrenal gland. It is possible that small amounts of oestrogens are also secreted by the normal adrenal cortex, but there is no direct evidence for this at present (Engel, 1962).

### Glucocorticoids

#### Cortisol

This substance, which is perhaps better known to the clinician as hydrocortisone, is the most important glucocorticoid secreted by the human adrenal cortex. Cortisone itself is not secreted by the human adrenal gland and is intrinsically biologically inactive as the effect of administered cortisone is dependent upon its conversion to cortisol which takes place mainly in the liver. This conversion is by no means complete and only about 50 per cent of an oral dose of cortisone is converted to the active hormone (Jenkins & Sampson, 1966).

Cortisol was first identified as the main adrenal hormone in human peripheral blood by Nelson *et al.* (1951), using the newly developed techniques of paper chromatography. Two years later, Bush & Sandberg (1953) confirmed their findings and showed that corticosterone was also present though in much smaller amounts, the ratio in man being about 8 : 1. This ratio is reversed in some animals and corticosterone is the main glucocorticoid in the rat.

In man, a healthy adult secretes between 5 and 30 mg of cortisol in 24 hours. During stress much larger amounts of cortisol are produced and it has been calculated that in the course of major surgical operations about 3–3.5 mg/hour may be secreted (Hume, Bell & Bartter, 1962). The maximum rate of cortisol secretion by normal man is even higher than this and is of the order of 10–20 mg/hour. The production of cortisol is regulated by the adrenocorticotrophic hormone (ACTH) of the pituitary gland.

Cortisol is not secreted at a constant rate throughout the 24 hours and adrenal activity normally rises to a peak between 4 a.m. and 8 a.m. and falls to very low levels during the night. It seems likely that this diurnal variation in adrenal activity is due to a diurnal rhythm in the secretion of ACTH from the pituitary gland (Ney *et al.*, 1963). The diurnal variation in cortisol secretion is reflected by the plasma cortisol level in the peripheral blood. The mean plasma cortisol level in normal persons between 6 a.m. and 8 a.m. is about 12  $\mu$ g/100 ml., and it falls progressively during the day to reach a level of less than 3  $\mu$ g/100 ml. between midnight and 3 a.m. This diurnal rhythm persists in blind persons and night workers, but it has been temporarily reversed in healthy volunteers whose day and night activities were completely reversed. It is normally preserved in chronically ill patients but is usually absent during acute illnesses, since stress of any kind will temporarily increase cortisol production and produce elevated plasma cortisol levels at any time

of the day or night. This diurnal rhythm is also absent, or markedly reduced, in patients with Cushing's syndrome (Eik-Nes *et al.*, 1955; Doe, Vennes & Flink, 1960).

When cortisol enters the bloodstream it becomes reversibly bound to plasma proteins. At concentrations below 20  $\mu\text{g}/100\text{ ml.}$  about 95 per cent is bound to a specific  $\alpha$ -globulin (transcortin), and only 5 per cent is in solution and freely diffusible throughout the body fluids. At higher concentrations all the binding sites on the transcortin are saturated and cortisol is then bound to albumin as well. The binding of cortisol to albumin is weaker than that to transcortin so that a greater proportion of the cortisol will be unbound at these higher concentrations and more will diffuse out into the tissues. Only the unbound fraction is biologically active, but as it diffuses out into the tissues it is replaced by cortisol released from the plasma proteins. The binding of cortisol to plasma proteins can be regarded as a buffering mechanism against rapid fluctuations in the level of biologically active hormone.

The plasma level of any substance represents a balance between its rate of secretion into the bloodstream and the rate at which it is removed. In most individuals the biological half-life of cortisol in the plasma is fairly constant at about 100 minutes so that the plasma cortisol level will usually reflect adrenocortical activity at about the time the blood is taken. This relationship between the plasma cortisol level and cortisol production is disturbed during pregnancy and in patients on oestrogen therapy. Oestrogens increase the amount of circulating transcortin and the capacity of this protein to bind cortisol is increased approximately fourfold. The total plasma cortisol level inevitably rises in these patients as a result of this increase in protein-binding, but the biologically active fraction remains almost unchanged (Mills *et al.*, 1960).

**Metabolism of Cortisol.** Most of the cortisol which diffuses out into the tissues is eventually returned to the bloodstream and destroyed in the liver. The liver contains a number of enzymes which attack different parts of the steroid molecule. Some of the cortisol is converted into tetrahydrocortisol and tetrahydrocortisone, and together these metabolites may account for as much as 35 per cent of the daily cortisol production. They are rapidly conjugated with glucuronic acid in the liver to form glucuronides and, since these substances are highly water-soluble and poorly bound to protein, they are readily excreted in the urine. Many of the other metabolites of cortisol are also conjugated with glucuronic acid, and it has been shown that between 57 and 90 per cent of an administered dose of  $^{14}\text{C}$ -labelled cortisol is excreted in the urine as glucuronides (Fukushima *et al.*, 1960). Conjugated cortisol metabolites are also excreted via the bile into the stools, and as much as 20 per cent of the daily cortisol production may be eliminated by this route.

Only a small fraction, amounting to not more than 2–3 per cent of the daily cortisol production, is normally excreted in the urine as unconjugated steroids. About 50  $\mu\text{g}/24\text{ hours}$  is in the form of unchanged cortisol, and the remainder consists of unconjugated cortisol metabolites.

**Physiological Effects.** Cortisol is the life-maintaining hormone of the adrenal cortex and plays an important role in the normal homeostasis of the body. Unfortunately, much of the experimental work with cortisol and its

synthetic analogues has been done under unphysiological conditions, so that it is difficult to disentangle the physiological effects of cortisol from those of cortisol excess.

There are many diverse physiological actions of cortisol, including those concerned with the internal distribution of water and electrolytes between the extracellular and intracellular compartments, the maintenance of blood pressure and glomerular filtration rate, and the renal regulation of water excretion (Beck & McGarry, 1962). Cortisol also stimulates gluconeogenesis and may under certain conditions reduce the utilization of ingested glucose. Hypoglycæmia sometimes complicates Addison's disease and hypopituitarism, and the enhanced sensitivity of these patients to exogenous insulin can be corrected by physiological doses of cortisol. Beck & McGarry (1962), however, consider the effects on carbohydrate metabolism to be of relatively minor importance.

Cortisol in excess has effects on carbohydrate, protein, and fat metabolism. In some patients it also has a mineralocorticoid action as well, producing sodium retention and an increased renal excretion of potassium. Gluconeogenesis is accelerated and there is an increased resistance to insulin. The deposition of glycogen in the liver is increased and the glucose tolerance curve may become abnormal. Excess cortisol has an "anti-anabolic" effect on protein synthesis and has been shown to suppress the incorporation of amino acids into the diaphragm of both intact and adrenalectomized rats. The deposition of fat is also increased and obesity is common in Cushing's syndrome and patients on corticosteroid therapy. Antibody formation and the inflammatory response to injury are both inhibited by large doses of cortisol or its analogues, and this accounts for the increased susceptibility to infection which is seen in patients on these drugs.

The precise mechanisms by which these actions are effected are still not clear, in spite of a vast amount of work on the subject. Pincus (1962) summarises the problems yet unsolved. He suggests the possibility that the steroids act (1) as co-factors or catalytic active centres in a nucleotide or protein complex, (2) as intervenors in a transport system operating at the cell surface or at the surface of intracellular enzyme sites, (3) to activate specific enzyme systems by any of several means, (4) as inducers of specific enzyme synthesis in target sites, or (5) as causes of enzyme adaptation to their presence. An analysis of the evidence regarding these possibilities is beyond the scope of this review.

### **Corticosterone**

Corticosterone is a relatively unimportant glucocorticoid in man. Peterson & Pierce (1960), using tritiated corticosterone, estimated that its secretion rate in normal adults ranged from 1.5 to 4.0 mg/24 hours, with a mean value of 2.3 mg/24 hours. They found that its secretion followed a similar diurnal rhythm to that of cortisol and also increased after the administration of ACTH. Normally the peak levels of corticosterone in the plasma are less than 2 µg/100 ml., and most of this is bound to the plasma proteins. Corticosterone is inactivated in the liver and its metabolites are excreted in the urine as conjugated steroids.

### Mineralocorticoids

Mineralocorticoids are, by definition, adrenal steroids which promote sodium reabsorption and potassium excretion by the kidney. Their role in the regulation of body sodium has been extensively reviewed by Slater (1964). In physiological amounts they act mainly on the distal tubules of the kidney, although there is some evidence to suggest that they may also influence sodium reabsorption in the proximal tubules. Mineralocorticoids increase the rate of exchange of sodium with potassium and hydrogen ions in the distal tubules, and this causes an increased excretion of potassium in the urine. A deficiency of these steroids in Addison's disease results in excessive sodium loss in the urine which eventually leads to severe sodium depletion.

### Aldosterone

Aldosterone is the main mineralocorticoid secreted by the human adrenal cortex, and was first isolated by Grundy, Simpson & Tait (1952). Aldosterone differs from cortisol and corticosterone in possessing an aldehyde group instead of a methyl group on carbon atom 18, and it also lacks the hydroxyl group on carbon atom 17 which distinguishes cortisol from corticosterone. Both cortisol and corticosterone have some mineralocorticoid activity but this is very weak compared to that of aldosterone.

Aldosterone is present only in very small amounts in the plasma, where it is mainly bound to albumin. These levels are so low that they can only be measured by very complicated isotopic techniques. Peterson (1964) has reported levels ranging from 0.002 to 0.015  $\mu\text{g}/100\text{ ml.}$  in normal subjects, and similar results have been reported by other workers. Aldosterone is broken down in the liver and its conjugated metabolites are excreted in the urine.

The control of aldosterone secretion is still a subject of much controversy. Large doses of ACTH will produce a transient increase in aldosterone secretion, but it seems unlikely that the pituitary plays a major role in the normal control of aldosterone secretion. Despite this uncertainty there is no doubt that the output of aldosterone from the adrenal glands is profoundly affected by changes in the sodium balance of the body. Under normal conditions the aldosterone secretion rate in adults, determined by an isotope dilution method, is of the order of 60–220  $\mu\text{g}/24\text{ hours.}$  Values as low as 25  $\mu\text{g}/24\text{ hours}$  have been reported in subjects in whom sodium retention has been produced by fludrocortisone (9  $\alpha$ -fluorohydrocortisone), whilst elevated levels of nearly 2,000  $\mu\text{g}/24\text{ hours}$  have been found in sodium-depleted patients. Salt depletion, hæmorrhage, dehydration and the administration of large amounts of potassium result in an increased secretion of aldosterone in normal subjects and, conversely, the output of aldosterone is suppressed by a high salt intake, expansion of the extracellular volume, and potassium depletion.

It is not clear how the adrenal cortex appreciates these changes in fluid and electrolyte balance, or how it adjusts its output of aldosterone in response to them. In recent years the role of the renin-angiotensin system in the control of aldosterone secretion has been the subject of intensive investigations: this is discussed in detail in Chapter 9. Renin is a proteolytic enzyme liberated by the cells of the juxtaglomerular apparatus of the kidney. It acts on an



$\alpha$ -globulin in the blood to form a decapeptide angiotensin I, which is then rapidly converted to the octapeptide, angiotensin II, by another circulating enzyme. There is no doubt that angiotensin II can stimulate the adrenal glands to produce aldosterone, but what part the renin-angiotensin system plays in the normal regulation of aldosterone secretion is still uncertain.

### **Mineralocorticoid Excess**

The main physiological action of the mineralocorticoids is on the renal tubules, but they affect the electrolyte content of other tissues as well. In excess they lower the sodium content of the sweat, saliva, and gastrointestinal juices, and this results in a fall in the sodium:potassium ratio in these secretions. The administration of large amounts of a mineralocorticoid to an adrenalectomized animal produces prolonged sodium retention, hypertension, and oedema. In an animal with intact adrenal glands this sodium retention only lasts a few days before the kidneys "escape" from the sodium-retaining effect of the mineralocorticoid. The reason for this "escape" is unknown. The expansion of the extracellular fluid is usually insufficient to produce visible oedema, but the blood pressure rises and the excessive potassium excretion in the urine continues.

In mineralocorticoid excess the potassium content of the skeletal muscles is depleted by the steady drain of potassium in the urine and is replaced by sodium and hydrogen ions. These electrolyte changes produce muscular weakness and may lead eventually to complete paralysis. The shift of hydrogen ions into the cells produces an intracellular acidosis and an extracellular alkalosis (Saunders *et al.*, 1960). The plasma potassium concentration usually falls to subnormal levels, but since potassium is mainly an intracellular ion the degree of hypokalaemia which is produced is only a rough guide to the amount of potassium lost from the muscles.

These effects of mineralocorticoid excess were first demonstrated 25 years ago by the administration of large amounts of deoxycorticosterone to normal dogs (Ferrebee *et al.*, 1941), and they occur spontaneously in patients with mineralocorticoid-secreting adrenal tumours. The majority of these patients have been shown to be secreting increased amounts of aldosterone, but the excessive renal excretion of potassium has not been reproduced to date by the prolonged administration of large amounts of aldosterone to normal subjects (Ross & Hurst, 1965). Evidence is accumulating that these tumours secrete more than one hormone, and it seems likely that the potassium depletion in these patients is due to a mixture of hormones and not to aldosterone alone.

### **Adrenal Androgens**

There is still considerable uncertainty about the exact nature and function of the androgenic hormones secreted by the human adrenal cortex. The situation is complicated since a number of the androgenic steroids which have been found in adrenal vein blood are freely interconvertible in the body. It is not yet clear whether all these substances are biologically active hormones or if some of them are only metabolites of active hormones which have retained part of the biological activity of the parent compounds. It seems likely, however, that the main androgenic hormones normally secreted by the human adrenal cortex are dehydroepiandrosteredione (DHEA), dehydro-

epiandrostenedione sulphate (DHEA sulphate),  $\Delta^4$ -androstenedione, and possibly  $11\beta$ -hydroxyandrostenedione (Prunty, 1966). The first two substances are freely interconvertible, whilst  $\Delta^4$ -androstenedione is derived from dehydroepiandrostenedione.  $\Delta^4$ -androstenedione is an interesting compound since it is readily converted into testosterone which is a much more potent androgen than any of those mentioned above. Testosterone has been found in the adrenal vein blood in some hirsute women (Burger, Kent & Kellie, 1964) and in a case of Cushing's syndrome (Hudson *et al.*, 1963), but there is no evidence at present to show that it is secreted in significant amounts by the normal adrenal gland.

A proportion of the  $\Delta^4$ -androstenedione which is produced is converted in the adrenal cortex to  $11\beta$ -hydroxyandrostenedione. This steroid has been found in human adrenal vein blood in higher concentrations than  $\Delta^4$ -androstenedione, but it is a much weaker androgen than its parent compound. Its importance lies in the fact that its metabolites retain the oxygen atom attached to carbon atom 11. This labels them as being derived from the adrenal cortex, since neither the testes nor the ovaries secrete 11-oxygenated steroids.

In the plasma the adrenal androgens are reversibly bound to plasma albumin and only about 5 per cent is non-protein bound and freely diffusable throughout the body water. Inactivation of these steroids takes place in the liver where some of the dehydroepiandrostenedione and all the  $\Delta^4$ -androstenedione is converted to androsterone and its isomer,  $\alpha$ -tiocholanolone. Dehydroepiandrostenedione and its metabolites are then conjugated either with sulphuric acid to form sulphates, or with glucuronic acid to form glucuronides.  $11\beta$ -hydroxyandrostenedione is converted to the 11-oxygenated derivatives of androsterone and  $\alpha$ -tiocholanolone, and these steroids are also conjugated with glucuronic acid. Both the sulphates and the glucuronides are readily water-soluble and all these metabolites are excreted in the urine as neutral 17-oxosteroids (more commonly, but chemically incorrectly, known as 17-ketosteroids).

Not all the neutral 17-oxosteroids in the urine are, however, derived from the adrenal androgens. Testosterone from the testes is also converted in the liver to  $\Delta^4$ -androstenedione and thence to androsterone and  $\alpha$ -tiocholanolone. Similarly, the ovaries may also produce some testosterone and  $\Delta^4$ -androstenedione. Finally, some cortisol molecules lose their side chain at carbon atom 17 and are converted into 11-oxygenated 17-oxosteroids in the liver. Normally less than 5 per cent of the daily cortisol production is metabolized in this way so that the contribution of these cortisol metabolites to the total urinary 17-oxosteroid excretion is usually negligible.

There is ample evidence that ACTH from the pituitary controls the rate of production of the adrenal androgens, but this is probably not the only controlling factor. The foetal adrenal cortex produces steroids which are qualitatively similar to those of the adult, but it produces relatively more androgens than glucocorticoids. Within two weeks of birth the output of androgens falls to a very low level and it does not rise again until the child is approaching puberty. Mills, Brooks & Prunty (1962) have shown that there is an unidentified factor in human pituitary extracts which augments the effect of ACTH on adrenal androgen production *in vitro*, and it is possible

that the rising output of adrenal androgens at puberty is due to synergism between ACTH and one of the gonadotrophins.

### **Physiological Effects of Androgens**

Synthetic steroids possessing androgenic properties are known to promote nitrogen retention and the synthesis of protein and it has been assumed for many years that this is one of the main functions of the adrenal androgens. There is, however, very little experimental evidence to support this idea and McSwiney and his colleagues (1964) were unable to demonstrate any anabolic effect when dehydroepiandrosteredione,  $\Delta^4$ -androsteredione,  $11\beta$ -hydroxy-androsteredione, or androsterone were administered by injection to hospital patients. Nevertheless, children secreting excessive amounts of adrenal androgens do grow faster than normal children, and their bone age, assessed radiologically, is often far in advance of their chronological age.

Androgen excess during childhood produces precocious puberty in the male and virilism in the female, but even in the normal female child the growth of body hair at puberty is dependent to some extent on the rising levels of adrenal androgens. Androgens also produce hypertrophy of the sebaceous glands at puberty, and the output of sebum in castrated males and in normal women appears to be largely dependent on the adrenal androgens.

### **PITUITARY-ADRENAL INTER-RELATIONSHIPS**

The production of the glucocorticoids and adrenal androgens is regulated by the adrenocorticotrophic hormone (ACTH) secreted by the anterior lobe of the pituitary gland. ACTH has a molecular weight of about 4,600 and is a single chain polypeptide made up of 39 amino acids. The sequence of the first 24 amino acids is common to ACTH obtained from the pig, sheep, cattle and man. Species differences involve only the amino acids in the N-25 to N-33 positions. The amino acid sequence from N-4 to N-11 in the ACTH molecule has melanophore-stimulating activity which can be enhanced by suitable additions to the chain at each end. Kappeler & Schwyzer (1961) have synthesized a polypeptide containing the first 24 amino acids of ACTH, and the adrenocorticotrophic effects of this synthetic compound have been demonstrated in man by Landon and his colleagues (1964). They found that similar and maximal rates of increase in the plasma cortisol level resulted from the intravenous infusion of 100  $\mu$ g/hour of the synthetic polypeptide and of 10 i.u./hour of highly purified porcine ACTH.

The rate of secretion of ACTH from the anterior pituitary is controlled by two mechanisms, of which the most important is the neural control over the synthesis and release of ACTH. This neural control is thought to be mediated by a specific chemical transmitter, corticotrophin-releasing factor (CRF), which is released into the portal system of veins which carry blood from the median eminence of the hypothalamus to the anterior lobe of the pituitary gland. This corticotrophin-releasing factor is a polypeptide with a molecular weight of about 1,000 and is chemically related to the posterior pituitary hormones, oxytocin and vasopressin (Saffran, 1962).

A wide variety of chemical agents, environmental conditions, and psychological phenomena can stimulate the release of ACTH from the pituitary by this neural mechanism. Surgery is a potent stimulus to the

pituitary-adrenal axis and the increased adrenocortical activity which accompanies surgical stress has been extensively investigated in recent years. Hume, Bell & Bartter (1962) found that complete transection of the spinal cord at T4 abolished the adrenocortical response to a major abdominal operation. Spinal analgesia, on the other hand, does not appear to prevent the normal response to surgery but may delay its onset. Morphine and barbiturates are known to inhibit the neural response to stress, and this inhibition probably accounts for the very low plasma cortisol levels which have been reported in surgical patients after pre-medication with omnopon and scopolamine, and following induction of anaesthesia with thiopentone (Mattingly & Tyler, 1965).

The secretion of ACTH from the pituitary is also regulated to some extent by the level of cortisol in the blood. A low plasma cortisol level stimulates the pituitary gland to secrete more ACTH. As the output of ACTH rises the adrenal cortex is stimulated to produce more cortisol and this, in turn, inhibits the further secretion of ACTH. In normal subjects this negative feed-back mechanism has a stabilizing influence on the plasma cortisol level, maintaining it within the physiological range throughout the day, but it must be subordinate to the neural control of ACTH secretion since it does not prevent the normal diurnal rhythm of pituitary-adrenal activity or interfere with the pituitary-adrenal response to stress. Estep and his colleagues (1963) have clearly demonstrated that the administration of high doses of cortisol or dexamethasone at the time of surgical operations does not abolish the normal pituitary-adrenal response to surgery. This feed-back mechanism is, however, very important in patients receiving prolonged corticosteroid therapy, for the secretion of ACTH will be inhibited during its administration and inevitably produces secondary adrenal atrophy.

ACTH is present only in minute amounts in human plasma, but methods have been developed for its estimation by biological assay (Lipscomb & Nelson, 1962; Ney *et al.*, 1963; Espiner, Beaven & Hart, 1963). Ney *et al.* (1963) found that the diurnal rise in the plasma cortisol level in normal subjects was associated with a mean plasma ACTH level of 0.25 m-u. per 100 ml. at 6 a.m., the diurnal fall in the plasma cortisol level being accompanied by a fall in the ACTH concentrations to a mean level of 0.11 m-u. per 100 ml. at 6 p.m. Elevated plasma ACTH levels, ranging from 0.42 to 4.7 m-u. per 100 ml., have been found in patients undergoing major surgical operations (Cooper & Nelson, 1962; Ney *et al.*, 1963). The normal human adrenal cortex appears to be remarkably sensitive to very small quantities of ACTH, and maximum adrenocortical activity is produced by plasma ACTH concentrations of the order of only 3.0 m-u. per 100 ml.

ACTH is rapidly destroyed in the body and the estimated half-life of ACTH in the plasma after intravenous injection is only about 10 minutes. *In vitro* experiments have shown that ACTH accelerates the conversion of cholesterol to pregnenolone in the perfused adrenal gland, and this results in an increased production of corticosteroids. The precise mechanisms by which this is brought about are still unknown.

ACTH has also been shown to have effects on other tissues besides the adrenal cortex, and these extra-adrenal actions have been extensively reviewed by Engel (1961). The direct effects of ACTH on fat and carbohydrate meta-

bolism may not be of any clinical importance, but its melanophore-stimulating activity is probably responsible for the increased skin pigmentation seen in Addison's disease, congenital adrenal hyperplasia, some patients with Cushing's syndrome, and patients being treated with high doses of ACTH for long periods. This melanophore-stimulating property of ACTH is due to a certain sequence of amino acids between N-4 and N-11 which is identical in the structure of the ACTH molecule and melanophore-stimulating hormone.

## ADRENAL FUNCTION TESTS

### Cortisol Production

Since cortisol is the main secretory product of the human adrenal cortex it is hardly surprising that many of the tests of adrenocortical function which have been developed are based on the measurement of cortisol or its metabolites in the plasma or the urine. The value and limitations of some of the tests in current use are discussed below.

### Cortisol Secretion Rates

Determined by an isotopic dilution technique, secretion rates provide an accurate measure of the total cortisol production over a period of 24 or 48 hours (Cope & Black, 1958). Unlike most other tests of adrenal function, their accuracy is not diminished at the lower levels of adrenal activity, and this method has proved invaluable in investigating adrenocortical function in man. At present it is unsuitable for routine clinical use since few laboratories have the time or technical skill required to do it, but it can be very helpful in investigating the difficult case and provides a reference standard against which to assess the validity of other simpler but less precise tests of adrenal function.

The method involves the introduction into the patient of a small tracer dose of radio-actively labelled cortisol and the collection of all the urine passed during the following 24 or 48 hours. Tetrahydrocortisone or tetrahydrocortisol is then isolated from the urine by paper chromatography and its specific activity is determined. The assumption is made that the tracer dose is metabolized by the same metabolic pathways as the endogenous cortisol, and that the same fraction of each will appear in the urine as these metabolites. The cortisol secretion rate is calculated from the equation,

Secretion Rate =  $\frac{\text{dose}}{M}$ , where the dose is the administered isotope-labelled

cortisol expressed as counts/minute, and M is the specific activity of the metabolite expressed as counts/minute per  $\mu\text{g}$  of metabolite.

Cope & Pearson (1965) have shown that the above equation is no longer valid when the blood urea is over 50 mg/100 ml., but they were able to modify it to allow for the incomplete excretion of the dose in patients with impaired renal function. The cortisol secretion rates found by these workers in 25 convalescent hospital patients ranged from 6.3 to 28.6 mg/24 hours, with a mean secretion rate of 16 mg/24 hours. Similar basal levels have been reported by others. There is a marked rise in the cortisol secretion rate after ACTH stimulation and Cope & Black (1958) found a mean secretion rate of

127 mg/24 hours in 12 subjects given ACTH, a figure almost 8 times the mean basal level.

### Plasma Cortisol Levels

Until quite recently the estimation of adrenal steroids in plasma has been difficult enough to deter all but the greatest enthusiasts. Most of the earlier methods for estimating cortisol in plasma were based either on the Porter-Silber reaction for "17-hydroxycorticoids" (Nelson & Samuels, 1952), or on the measurement of the fluorescence of the individual steroids after their separation by chromatography (Sweat, 1954; Lewis, 1957; Bondy *et al.*, 1957). The only method which has been widely used for research purposes is Peterson's modification of the Porter-Silber reaction (Peterson, Karrer & Guerra, 1957). Even this method requires at least 5 ml. of plasma for a single estimation and is too laborious for routine clinical use.

An advance of major clinical importance was made when simple but effective methods based on fluorescence were developed for estimating cortisol in human plasma (De Moor *et al.*, 1960; Mattingly, 1962). These methods depend on the fluorescence of certain steroids in concentrated sulphuric acid and only require 2 ml. of plasma for each estimation. The substances which are estimated are steroids with both the  $\Delta^4$ -3 ketone configuration and also an hydroxyl group on carbon atom 11 which is

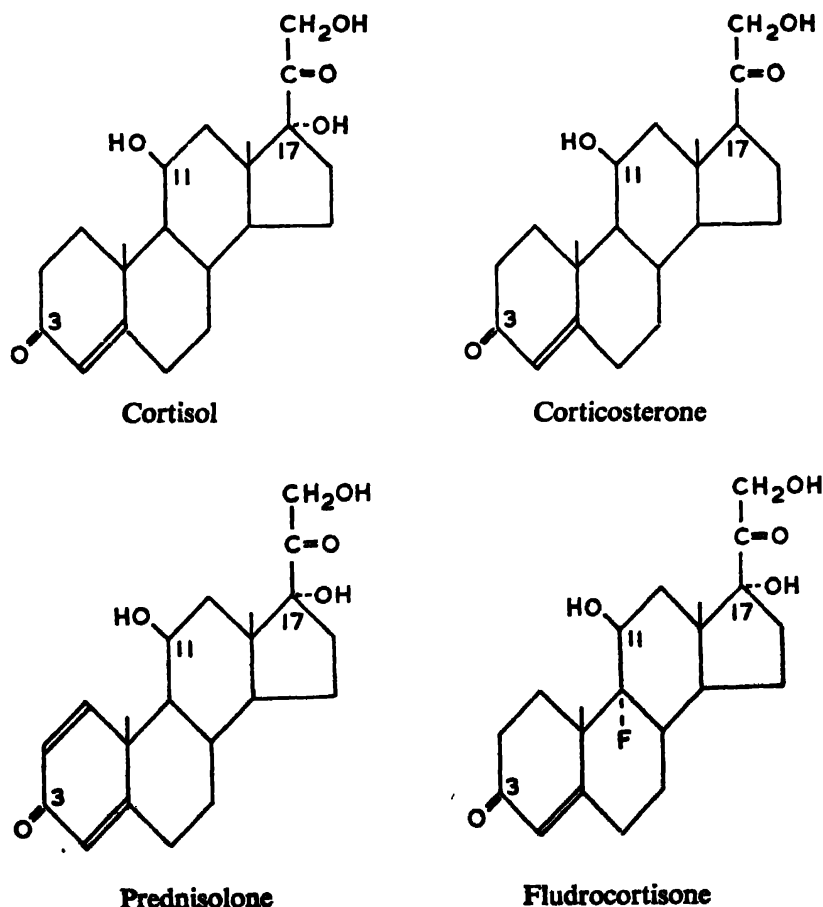


FIG. 5.1. The formulæ of cortisol, corticosterone, and two synthetic analogues of cortisol.

unique to steroids with an adrenal origin. These steroids are known as 11-hydroxycorticoids. The main 11-hydroxycorticoid in human plasma is cortisol, but a small amount of corticosterone is present as well (Fig. 5.1).

These methods are fairly specific, for De Moor and his colleagues (1962) tested 38 steroids and found that only cortisol, corticosterone, 20 $\beta$ -hydroxycortisol and 21-deoxycortisol produced any fluorescence by their method. The last two steroids are not normally present in human plasma, but 21-deoxycortisol has been detected in the plasma of a patient with congenital adrenal hyperplasia (Wieland *et al.*, 1965). The introduction of another unsaturated bond between carbon atoms 1 and 2 in the cortisol molecule, as in prednisolone, or a fluorine atom at carbon 9, as in 9 $\alpha$ -fluorohydrocortisone (fludrocortisone), abolishes the fluorescence (Fig. 5.1). All the synthetic analogues of cortisol and cortisone possess one or both of these modifications so that none fluoresces and it is possible to measure adrenocortical activity in patients on these drugs. Cortisone itself does not fluoresce, but it is converted in the body into cortisol.

Using these fluorimetric methods it is possible to complete 6 estimations in 1½ hours, and this makes possible a new attitude to the clinical elucidation of adrenal and steroid problems, for answers to these problems can often be obtained the same day. Plasma 11-hydroxycorticoid estimations only reflect

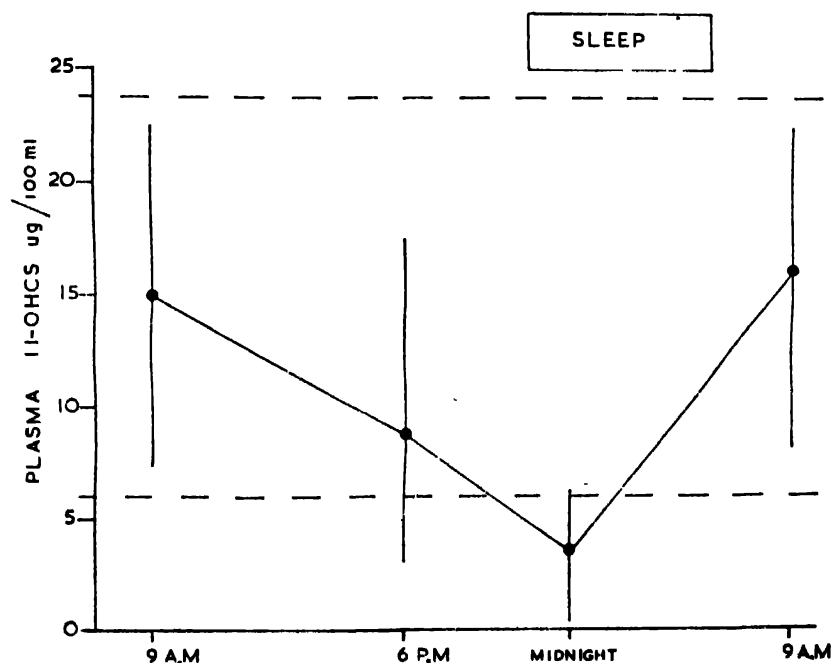


FIG. 5.2. Mean diurnal variation of plasma 11-hydroxycorticoid levels in 24 normal subjects. The vertical lines indicate the range of observations. The horizontal dashed lines show the normal range between 9 a.m. and 10 a.m.

adrenocortical activity at the time the blood is taken and it is important to take this into account when interpreting the results. Basal levels should be measured in the morning to avoid the normal diurnal variation in plasma cortisol levels, which may be large. Mattingly (1963), for example, found that the mean plasma 11-hydroxycorticoid level between 9 a.m. and 10 a.m. in

100 convalescent hospital patients was  $14.7 \mu\text{g}/100 \text{ ml.}$ , the range being  $5.7\text{--}23.7 \mu\text{g}/100 \text{ ml.}$  At midnight, however, the mean plasma 11-hydroxycorticoid level in 24 of these patients had fallen to  $3 \mu\text{g}/100 \text{ ml.}$ , the range now being  $0\text{--}6.2 \mu\text{g}/100 \text{ ml.}$  (Fig. 5.2).

These estimations measure all the "free", or unconjugated, 11-hydroxycorticoids in the plasma. Most of this is reversibly bound to the plasma proteins and hence is physiologically inactive, and it is only the small diffusible fraction which determines the cortisol concentration in the tissues. This can be separated by ultra-filtration through a cellophane membrane, but the cortisol concentration in the ultra-filtrate is too low to be measured accurately by fluorimetry. This difficulty can be overcome by adding isotope-labelled cortisol to the plasma before ultra-filtration and measuring the proportion of the radio-activity which appears in the ultra-filtrate (Mills *et al.*, 1960), but this technique is too laborious for routine clinical use.

The total plasma 11-hydroxycorticoid level will usually be a reliable measure of adrenocortical activity except during pregnancy and oestrogen therapy when the protein-binding is markedly increased. High levels in these patients do not necessarily indicate increased adrenal activity. Metcalf & Beaven (1963), for example, found elevated plasma 11-hydroxycorticoid levels in women taking contraceptive tablets and this possibility should be borne in mind if high levels are found in otherwise healthy women. The only other drugs known to interfere with these estimations are mepacrine and aldactone.

### Conjugated Corticosteroids in Urine

Cortisol metabolites are mainly conjugated with glucuronic acid and several adrenal function tests have been developed which are based on the group estimation of these conjugated steroids in the urine. One method which has been widely used is that developed by Gibson & Norymberski (1954) for the estimation of 17-ketogenic steroids. This nomenclature is chemically incorrect and these substances are now called 17-oxogenic steroids. 17-oxogenic steroids are steroids whose side-chain on carbon atom 17 can be removed by oxidizing agents to form 17-oxosteroids. The latter, which were previously (but chemically incorrectly) called 17-ketosteroids, are then estimated by the Zimmerman reaction. This method also measures any 17-oxosteroids already present in the urine so that these must be determined separately and the result subtracted from the total.

The need to perform two separate estimations on each urine sample was soon overcome by modifying the method so that the 17-oxosteroids were first destroyed by preliminary treatment of the urine with borohydride (Appleby *et al.*, 1955). All the steroids estimated by this modified method have an hydroxyl group on carbon atom 17 and are therefore known as 17-hydroxycorticoids. In most instances the two methods give similar results, but the 17-hydroxycorticoid method tends to give slightly higher values with some urines. The estimation of the 17-hydroxycorticoids is the method of choice since it takes less time and is more precise than the estimation of the 17-oxogenic steroids. The normal range is  $5\text{--}18 \text{ mg}/24 \text{ hours}$  in adult females, and  $6\text{--}22 \text{ mg}/24 \text{ hours}$  in adult males. These methods are of little value in assessing adrenocortical hypofunction during childhood, since the levels are



so low below the age of puberty that only gross divergencies from normal can be detected.

Both these methods share a number of shortcomings which are not always appreciated by clinicians. The substances estimated are steroid metabolites of cortisol and its inactive precursors, and these methods do not distinguish between them. Elevated levels do not necessarily indicate an increased production of cortisol but may be due to a block in cortisol synthesis, resulting in an increased output of its precursors. The urinary excretion of these conjugated steroids is dependent on renal function and may be seriously affected by alterations in the glomerular filtration rate. When renal function is impaired there is retention of these conjugated steroids in the body and a reduced output in the urine. Neither of these methods is sufficiently accurate to distinguish between the low levels found in many debilitated patients from those occurring in patients with adrenal hypofunction.

Drugs administered to patients seem not to interfere with these estimations, with the exception of meprobamate, but a serious source of error is the presence of glucose in the urine. Glucose prevents the oxidation of 17-oxogenic steroids to 17-oxosteroids, and serious under-estimation will occur unless steps are taken to remove the glucose before assay.

These methods have been widely used in the clinical investigation of adrenal disorders, but the not infrequent finding of normal or only slightly elevated levels in patients with Cushing's syndrome has thrown some doubt on their validity as a measure of cortisol production. Recently, Cope & Pearson (1965) have compared the results of 17-oxogenic steroid assays with the actual cortisol secretion rates in patients with a wide range of adrenocortical activity. They came to the rather surprising conclusion that there was no correlation at all between these methods over the normal range, or in the range just above it where so many of the equivocal results are found. They did find, however, that there was some correlation between urinary 17-oxogenic steroid excretion and the cortisol secretion rate when the latter was well above the normal range, and the values then obtained were roughly 40-50 per cent of the total cortisol production.

There are other methods for the estimation of conjugated steroids in urine but they have not been shown to have any advantages over the methods developed by Norymberski. Most of the methods for estimating individual conjugated steroids in the urine are too laborious for routine clinical use, but relatively simple methods for the estimation of pregnanetriol have been developed which are of value in the diagnosis of congenital adrenal hyperplasia (Fotherby & Love, 1960).

### Urinary Cortisol Estimations

Less than 0.5 per cent of the cortisol produced by the adrenal cortex is normally excreted unchanged in the urine. Nevertheless, this small amount can be measured by suitable techniques and these estimations are of particular value in the diagnosis of Cushing's syndrome. Cope & Black (1959), using paper chromatography, found that the normal cortisol excretion varied from less than 10  $\mu\text{g}$  to about 100  $\mu\text{g}/24$  hours, with a mean of 43  $\mu\text{g}$ . The mean rise in urinary cortisol output in 12 cases of Cushing's syndrome was

8.5 times the normal and was the most sensitive index of increased adrenal activity in their patients. Similar results have been reported by other workers.

### **Urinary 11-hydroxycorticoid Estimations**

Mattingly and his colleagues (1964) have recently described a rapid screening test for adrenocortical function which is based on the fluorescence of free (unconjugated) 11-hydroxycorticoids in urine. They found a good correlation between these estimations and the cortisol secretion rates determined simultaneously in 54 patients with a wide variety of disorders. This correlation was, in fact, much closer than that found when the urinary excretion of 17-oxogenic steroids is compared with the cortisol secretion rate. Most of the fluorescence in the urine extracts appears to be produced by free cortisol and its metabolite, 20-hydroxycortisol.

The attraction of this urinary fluorescence method is essentially its speed and relative simplicity. The levels in 42 convalescent hospital patients ranged from 78–372  $\mu\text{g}/24$  hours when expressed as cortisol equivalents. By contrast, the levels in 20 patients with Cushing's syndrome ranged from 400–7,000  $\mu\text{g}/24$  hours. The highest value encountered was in a patient with Cushing's syndrome whose hyperplastic adrenals were being stimulated with ACTH. Her excretion reached 21,400  $\mu\text{g}/24$  hours.

The synthetic steroids do not fluoresce so that this method can be used to measure the degree of adrenocortical suppression produced by these drugs. Glucose in the urine does not affect these estimations, and the only drugs administered to patients which are known to interfere with them are aldactone and mepacrine. Mepacrine can produce abnormally high levels of fluorescence in the urine for as long as 30 days after stopping its administration (Freitase Costa, Sobrinho & de Oliveira, 1965).

### **ACTH Stimulation Tests**

A large number of ACTH tests have been described which differ in the dose and preparation of ACTH which is employed, in the route of administration and duration of the test, and in the method used to determine the adrenocortical response to stimulation. Tests based on the intravenous infusion of ACTH involve the risk of severe allergic reactions and have not been widely adopted. This potential hazard is largely avoided if ACTH is given by intramuscular injection, and the use of ACTH gel by this route provides a fairly prolonged stimulus to the adrenal glands.

The estimation of plasma 11-hydroxycorticoids provides a quick and relatively simple measure of the adrenal response to ACTH gel, the peak levels in normal subjects being reached between 4 and 6 hours after the injection (Fig. 5.3). Blood is taken for a basal plasma 11-hydroxycorticoid estimation between 9 a.m. and 10 a.m. and the patient is then given an intramuscular injection of 50 units of ACTH gel. A further blood sample is taken 5 hours later and the response is measured by the rise in the plasma 11-hydroxycorticoid level over the 5 hours (Mattingly, 1963). There is a wide range of response to this test in patients with intact adrenal glands, and in 88 hospital patients studied by the author the increases have ranged from 19.2 to 107.7  $\mu\text{g}/100$  ml., with a mean rise of 44.8  $\mu\text{g}/100$  ml. This test can be completed within a few hours of admitting a patient to hospital.

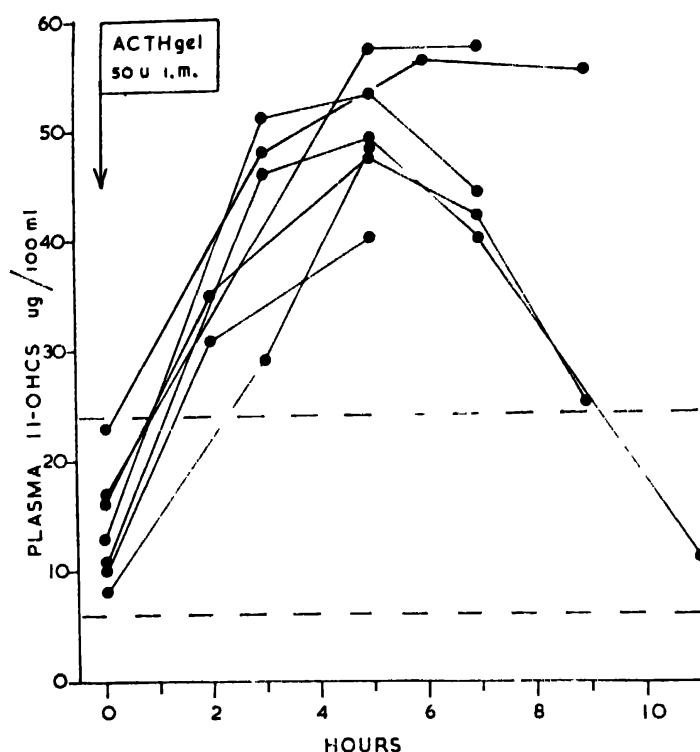


FIG. 5.3. Plasma 11-hydroxycorticoid response to a single intramuscular injection of 50 units of ACTH gel in 7 normal subjects. The horizontal dashed lines show the normal range between 9 a.m. and 10 a.m.

An even quicker screening test which can be used in an out-patient clinic has been described by Maynard and his co-workers (1966). The patient is given a single intramuscular injection of 25 units of aqueous ACTH, and the plasma 11-hydroxycorticoid level is measured immediately before and one hour after the injection. The mean rise in the plasma 11-hydroxycorticoid level in their 21 control subjects was  $24.7 \mu\text{g}/100 \text{ ml.}$ , the range being  $11.3$  to  $47.8 \mu\text{g}/100 \text{ ml.}$  No response was seen in 10 patients with primary adrenal insufficiency.

Wood and his co-workers (1965) have described a similar test which can also be used in an out-patient clinic. They measured the rise in the plasma 11-hydroxycorticoid level 30 minutes after a single intramuscular injection of  $250 \mu\text{g}$  of a synthetic polypeptide  $\beta^{1-24}$  corticotrophin (Synacthen, Ciba), equivalent to 25 units of natural hormone. The mean rise in their 66 normal subjects was  $16.7 \mu\text{g}/100 \text{ ml.}$ , the range being  $7.5$  to  $27.5 \mu\text{g}/100 \text{ ml.}$  One patient who failed to respond to this test was subsequently shown to have Addison's disease. This synthetic polypeptide has certain advantages over ACTH extracted from the pituitary glands of animals. As it is a pure compound it can be assayed by weight and is not contaminated by foreign proteins. This purity, and its shorter amino acid sequence which is common to all known forms of ACTH, decrease the likelihood of hypersensitivity reactions. The main disadvantage of this synthetic polypeptide is its short duration of action which is over within 4 hours of a single intramuscular injection. This limits its use in therapy.

Both these rapid ACTH tests give reproducible results and have greatly

facilitated the screening of patients with suspected Addison's disease. The use of the synthetic polypeptide reduces the risk of hypersensitivity reactions and it should be used in preference to the natural hormone in all patients with an allergic history. The prolonged action of ACTH gel, on the other hand, provides a greater stimulus to the adrenal glands and is more useful in evaluating adrenocortical function in patients with suspected secondary adrenal atrophy, and in restoring this to normal if impaired.

Although more laborious to estimate than plasma levels the rise in the urinary excretion of 17-oxogenic steroids or 11-hydroxycorticoids can also be used to measure the adrenal response to intramuscular ACTH gel. Brooks and his colleagues (1963), for example, have found that the urinary 17-oxogenic steroid excretion rises in normal subjects to levels between 40–90 mg/24 hours after the administration of a potent sample of ACTH gel in a dose of 20 units twice a day for 4 days. The free urinary 11-hydroxycorticoids are an even more sensitive index of adrenal activity than the urinary 17-oxogenic steroids, and it is not uncommon to get a 20-fold rise over the basal levels in normal subjects given ACTH gel in a dose of 40–50 units twice a day for 3 days. Urine is collected on one control day and on the third day of ACTH administration. It is advisable to give the ACTH gel by deep intramuscular injection in a dose of at least 40 units twice a day to ensure maximum adrenal stimulation.

#### **Tests of Pituitary-adrenal Function**

ACTH stimulation tests only indicate the degree of adrenal atrophy which may be present and give no information about the ability of the pituitary gland to produce and secrete ACTH. Secondary adrenal atrophy will be present only when the pituitary secretion of ACTH is grossly impaired, and less severe degrees of hypopituitarism will be missed if too much reliance is placed on the results of ACTH tests alone. These patients may be at risk from acute adrenal insufficiency if they are subjected to surgical operations in the mistaken belief that their pituitary-adrenal axis is functioning normally. This particularly applies to patients who have been on corticosteroid therapy, since these drugs inevitably suppress pituitary-adrenal function during their administration and the resultant impairment of adrenocortical function may persist for months or even years after the treatment has been stopped.

A variety of methods have been proposed for testing pituitary and adrenal function in these patients, but none are entirely satisfactory. Ideally, the procedure should test the entire hypothalamic-pituitary-adrenal system and should be simple enough to apply to routine clinical situations. It should also be reproducible and free from any undesirable side-effects. Stressful situations, such as insulin-induced hypoglycæmia and fever produced by bacterial pyrogens, are potent stimuli to the neural mechanism controlling ACTH release, but they have not been widely used because the response to a standard dose is unpredictable and they are not without risk to the patient. For example, it is usually necessary to lower the blood glucose level to less than 30 mg/100 ml. with intravenous insulin before there is any significant rise in the plasma cortisol level. It is almost impossible to select the correct dosage of insulin to produce this degree of hypoglycæmia in every patient without running the risk of producing severe hypoglycæmia in some of them.

A strong natural stimulus to ACTH release is provided by an abnormally low plasma cortisol level, which occurs during the recovery of the pituitary-adrenal system after corticosteroid administration. Robinson, Mattingly & Cope (1962) tested the integrity of the pituitary-adrenal axis after prolonged corticosteroid therapy by following the spontaneous rise in plasma cortisol concentrations after the sudden cessation of treatment. If this system is intact a rise to normal levels occurs within 48 hours of the last dose. This is a quick and relatively simple test which has proved invaluable in picking out the occasional patient whose pituitary-adrenal function is grossly impaired by corticosteroid therapy, and it has also been of value in determining whether the pituitary has been completely destroyed by surgery or radioactive seeds in the treatment of carcinoma of the breast. However, the ability to maintain a normal plasma cortisol level following steroid withdrawal does not necessarily imply that the pituitary is capable of secreting increased amounts of ACTH during stress, nor is it any guarantee that the adrenal glands are capable of responding to increased levels of ACTH by producing more cortisol. This investigation is useful only where patients have received corticosteroid therapy, and so has only limited application. For more general use, two other tests, the metyrapone test, and the vasopressin test, have been devised to check the integrity of the pituitary-adrenal system.

### **Metyrapone (Metapirone) Tests**

The discovery that the insecticide DDT produced adrenal atrophy in animals led to a search for other compounds which might interfere with adrenal function. A number of substances have now been synthesized which have this property, but the only one which has been widely used is metyrapone (2-methyl-1, 2-bis(3-pyridyl)-1-propanone:metapirone) which produces a selective inhibition of the activity of the enzyme,  $11\beta$ -hydroxylase, in the human adrenal cortex (Liddle *et al.*, 1958; Jenkins *et al.*, 1958). Inhibition of the activity of this enzyme by metyrapone interferes with the production of cortisol and corticosterone, and their 11-deoxy precursors are produced instead. The plasma cortisol level falls rapidly to very low levels and the pituitary promptly secretes more ACTH, since the normal "feed-back" control of ACTH release is thus removed. The elevated ACTH levels in the blood accelerate adrenal steroid synthesis and increasing amounts of 11-deoxycortisol (Reichstein's compound S) and 11-deoxycorticosterone (DOC) are produced. As a result the urinary excretion of 17-oxosteroids and 17-oxogenic steroids rises during the administration of metyrapone, the increase in the latter fraction being largely due to the presence in the urine of abnormal amounts of the tetrahydro derivative of compound S.

Liddle and his colleagues (1959) recognized the potential value of this drug as a means of indirectly testing the ability of the pituitary to secrete ACTH, and were the first to use it as a test of "pituitary reserve" in clinical practice. Metyrapone is a relatively non-toxic drug and can be administered either by continuous intravenous infusion or in divided doses by mouth. The oral route is the most convenient method of administration and it should be given in a dose of 750 mg every four hours for six doses. Daily doses of 4.5 g of metyrapone usually produce 95 per cent inhibition of the enzyme system but smaller doses than this are less effective in blocking

cortisol production (Cope, Dennis & Pearson, 1966). There is little point in giving larger amounts or prolonging the test for longer than 24 hours. The effect of a single dose is over in four to five hours so that it is necessary to give it four-hourly throughout the 24 hours. The commonest side-effect of metyrapone is a sensation of lightheadedness or giddiness which lasts for about half an hour after taking the tablets. The incidence of this can be reduced by giving the tablets after food, or with a glass of milk, and keeping the patient in bed during the test. There is always the possibility of precipitating acute adrenal insufficiency in patients given metyrapone, and this should be considered if patients complain of headache, drowsiness, abdominal pain or nausea.

The pituitary-adrenal response to metyrapone is most conveniently determined by measuring the urinary excretion of 17-hydroxycorticoids (or 17-oxogenic steroids) on the day before, during, and on the day after metyrapone administration. Peak levels are sometimes found on the day of administration but usually occur on the following day. The response is measured by the maximum rise in steroid excretion on either of these two days over the control day level. Normally this rise will be greater than 10 mg in the 24 hours. For example, Landon, James & Stoker (1965) performed this test in 29 control subjects and found a mean rise of 23.9 mg/24 hours, the range being 10.8–37.6 mg/24 hours.

A poor response to this test does not necessarily imply that the pituitary is incapable of secreting more ACTH, for the adrenal itself may be atrophied and unable to respond. Whenever an impaired response is obtained to this test it should be followed by an ACTH stimulation test to exclude this possibility. Corticosteroids or exogenous ACTH will obviously interfere with the metyrapone test by suppressing ACTH secretion from the pituitary and should be discontinued for at least three days beforehand.

Metyrapone tests have been widely used to assess the ability of the pituitary to secrete ACTH, but they only provide a measure of the integrity of the normal feed-back control and under conditions of stress other mechanisms appear to influence ACTH secretion (Estep *et al.*, 1963). An impaired response to this test does not necessarily mean that the patient will not respond normally to surgical stress.

### Vasopressin Tests

Vasopressin is chemically related to the corticotrophin-releasing factor (CRF), and when vasopressin is administered to man in adequate dosage it produces a rise in the plasma cortisol level which is apparently due to a direct action on the anterior pituitary. This response can be completely abolished by the prior administration of a single dose of 1.5 mg of dexamethasone two hours beforehand, so that it is unlikely that it is due to direct stimulation of the adrenal cortex (Clayton *et al.*, 1965). Morphine also abolishes the rise in the plasma cortisol level following an injection of vasopressin, but does not prevent a normal response to intramuscular ACTH, again suggesting that the effect of the vasopressin is a direct one on the release of ACTH by the pituitary (Gwinup, 1965a).

Gwinup (1965b) has developed a simple test using synthetic lysine-vasopressin to test the ability of the pituitary to secrete ACTH. The test is

carried out in the early afternoon and the patient is given 10 pressor units of synthetic lysine-vasopressin (Sandoz) intramuscularly. Blood is drawn from an antecubital vein immediately before and one hour after the injection of vasopressin, and assayed for plasma 11-hydroxycorticoids. The normal response to this test is a prompt and consistent rise in the plasma 11-hydroxycorticoid level which reaches a peak about one hour after the injection. Gwinup found that this peak level in 22 normal subjects was more than twice the control value, but there was no response to equivalent volumes of intramuscular saline. A few patients experience minimal bowel cramps of a transient nature, but otherwise the procedure appears to be relatively simple and safe.

A similar but more complicated test, using a continuous intravenous infusion of lysine-vasopressin, has been recently described by Landon, James & Stoker (1965). These workers studied 16 patients with hypothalamic or pituitary disorders and came to the conclusion that their results were consistent with an ACTH-releasing action of lysine-vasopressin. However, in four patients with hypothalamic disorders the adrenal response to ACTH and vasopressin was normal although the responses to metyrapone and to insulin-induced hypoglycæmia were impaired. These findings suggested that functional pituitary tissue was present but that hypothalamic control of the pituitary was disturbed. One of these patients later developed acute adrenal insufficiency during an air encephalogram so that a normal vasopressin test does not necessarily mean that a patient will produce an adequate adrenal response to stress. This dissociation is compatible with the view that these procedures test different levels of the hypothalamic-pituitary-adrenal axis, and it seems likely that only stressful stimuli such as hypoglycæmia test the integrity of the entire axis.

Although the usefulness of the vasopressin test is limited in this way, the ease with which it can be carried out makes it a worthwhile procedure. It is clear that an impaired response to this test indicates a defective pituitary-adrenal axis and these patients will require corticosteroid cover during stressful situations.

### **Aldosterone Production**

The daily production of aldosterone is only one-hundredth that of cortisol, and the measurement of such minute amounts presents great difficulties. No methods are available at present for routine clinical use. Methods have been developed in recent years for the estimation of aldosterone secretion rates, plasma aldosterone levels, and urinary aldosterone excretion, but all these methods require considerable skill and experience and are limited to a few research laboratories. Aldosterone secretion rates are determined by isotope dilution, using similar techniques to those employed for cortisol (Cope, Nicolis & Fraser, 1961). High values have been found in sodium-depleted patients, in patients with aldosterone-secreting tumours, and in some patients with severe hypertension. Chemical methods for the estimation of aldosterone in urine are of limited value, for the proportion of the daily aldosterone production which is estimated by these methods is not constant and can vary from about 3 to 11 per cent in different patients. The normal range in adults is about 2–15  $\mu\text{g}/24$  hours.

### Androgen Production

The conjugated metabolites of the adrenal androgens are 17-oxosteroids, and form a major part of the total 17-oxosteroid excretion in the urine. Group methods for the estimation of the urinary 17-oxosteroids have been widely used for many years, but they are a relatively crude index of adrenal androgen production (Goldzieher & Axelrod, 1962; Ernest *et al.*, 1964). These methods do not distinguish between the metabolites of the androgens secreted by the adrenal cortex and those derived from the testes or the ovaries. More specific methods for the estimation of individual androgens and their metabolites in blood and urine are too laborious at present for routine clinical use.

During the first two weeks of life the urinary 17-oxosteroid excretion may reach 5 mg/24 hours, but after this it falls rapidly to less than 1 mg/24 hours until the child is between six and ten years old. From then on it slowly rises to reach adult levels after puberty. Maximum excretion is reached in both sexes around the age of 20, and there is a slow decline in 17-oxosteroid levels after the age of 50. The normal range in young adults is 6–26 mg/24 hours in men and 4–17 mg/24 hours in women. The mean value in men is about 5 mg a day higher than the mean value in women, and this difference is largely due to the additional 17-oxosteroids derived from the testicular androgens.

Low 17-oxosteroid levels are often found in chronically ill patients, particularly if renal function is impaired, so that these estimations will be misleading if they are used as the sole criterion for diagnosing adrenal hypofunction. Similarly, it has been known for many years that the normal response of the urinary 17-oxosteroids to ACTH stimulation is very variable, and a failure to respond to ACTH is not diagnostic of adrenal failure.

### HYPOADRENALISM

Damage to either end of the pituitary-adrenal axis can result in adrenal hypofunction. Both cortisol and aldosterone are essential to life, but in most patients the signs and symptoms of acute adrenal insufficiency are due to the failure of the adrenal glands to meet the increased demand for cortisol during stress. Aldosterone deficiency only occurs in primary adrenal disorders. The main causes of hypoadrenalism are primary adrenal failure, damage to the ACTH-releasing mechanisms by disease of the hypothalamus and pituitary gland, and inhibition of the pituitary-adrenal axis by corticosteroid therapy. Other causes include inhibition of steroid synthesis by drugs and destruction of the pituitary or adrenal glands by surgery or irradiation.

Primary adrenal failure is usually due to destruction of both adrenal glands. Less commonly, it is due to genetically determined defects of cortisol synthesis. A fascinating but rare disorder of selective hypoaldosteronism has also been described which is not associated with any impairment of cortisol production (Posner & Jacobs, 1964).

### Addison's Disease

**Ætiology.** Addison's disease is a chronic disorder which is due to the progressive destruction of both adrenal glands. In about 50 per cent of cases



this is caused by tuberculosis. In most of the non-tuberculous cases there is a progressive atrophy or necrosis of the adrenal glands which is accompanied by a marked lymphocytic infiltration. The similarity between this histological picture and that found in the thyroid in Hashimoto's disease has prompted a number of workers to look for evidence of an auto-immune process, and adrenal antibodies have been found in no less than 51 per cent of the 71 patients in one series (Blizzard & Kyle, 1963). Whether these antibodies are the cause or the result of the adrenal destruction is still uncertain. On rare occasions Addison's disease is caused by secondary amyloidosis, and in endemic areas it is sometimes due to mycotic infections.

The severity of the disease and the mode of presentation will largely depend on the rapidity with which the glands are destroyed. If aldosterone deficiency occurs early in the course of the disease the symptoms and signs of sodium depletion may dominate the clinical picture. Some patients develop a craving for salt, but this is not common. As the disease progresses the pituitary responds to a falling plasma cortisol level by secreting excessive amounts of ACTH. These elevated ACTH levels stimulate the adrenal remnant to a high degree of activity and enable it to maintain a more or less normal output of cortisol for some time. There will, however, be no adrenal reserve in these patients and acute adrenal insufficiency may be precipitated by a mild upper respiratory infection or minor trauma.

**Clinical Features.** All the important clinical features of this disease were first described by Thomas Addison in 1855. A severe case may be diagnosed at a glance, but many patients with this disease have a period of ill-health lasting months and sometimes years before the diagnosis of chronic adrenal failure is suspected. The percentage incidence of the main presenting features of this disorder in 21 patients studied by the author are shown in Table 5.I.

TABLE 5.I  
PERCENTAGE INCIDENCE OF MAIN FEATURES OF ADDISON'S DISEASE  
IN 21 PATIENTS

	%		%
Excessive tiredness	100	Abdominal pain	42
Generalized pigmentation	95	Previous tuberculous infection	42
Weight loss	75	Postural hypotension	38
Anorexia	72	Diarrhoea	38
Nausea and vomiting	72	Dyspnoea on exertion	38
Low blood pressure	67	Muscle cramps	29
(systolic < 105 mm Hg)		Old tuberculous lesion on chest	24
Mental changes	62	X-ray	
Buccal pigmentation	48	Adrenal calcification	14

The biochemical abnormalities which are found in the blood in Addison's disease are a low serum sodium concentration, a raised serum potassium concentration, a metabolic acidosis, and a raised blood urea. These abnormalities are almost invariably found, to a lesser or greater degree, in patients presenting in an adrenal crisis, but they may be absent in the more chronic cases. The 17-oxogenic steroid excretion in these latter patients is not infrequently within the normal range (Fig. 5.4). Cortisol deficiency impairs the ability of the kidneys to excrete a water load and the low serum sodium

concentration is partly due to overhydration of the plasma. Water excretion tests have been used in the past as a screening test for hypoadrenalism, but they can be very misleading. A normal response does not exclude Addison's disease since the adrenal remnant may still be secreting some cortisol, whilst abnormal responses are found in other disorders such as cirrhosis of the liver, congestive cardiac failure, myxœdema, obesity, renal disease, rheumatoid arthritis, steatorrhœa, and after dehydration.

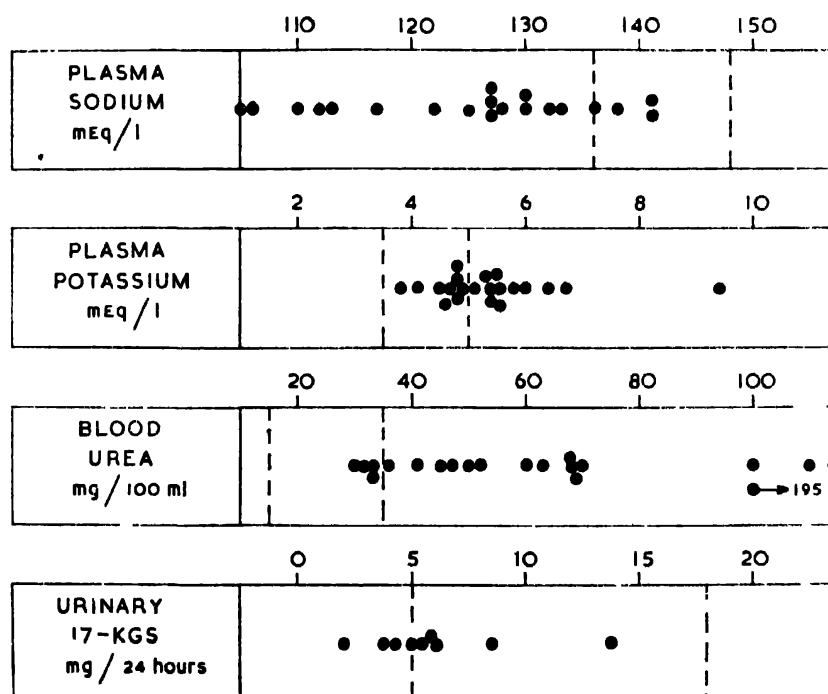


FIG. 5.4. Plasma sodium, potassium and blood urea concentrations in 21 patients with untreated Addison's disease. Urinary 17-oxogenic steroids were estimated in only 9 of these patients (as 17-KGS).

**Differential Diagnosis.** Many of the features of Addison's disease, including the buccal pigmentation, are found in other conditions. These include cirrhosis of the liver, Crohn's disease, hæmochromatosis, pernicious anæmia, chronic renal failure, steatorrhœa, thyrotoxicosis, busulphan therapy, and various forms of malignant disease. The diagnosis of Addison's disease can only be made with certainty in life by demonstrating the inability of the adrenal glands to respond to adequate ACTH stimulation. Normal adrenal function tests in the unstimulated patient may be misleading, since the adrenal remnant in some patients with Addison's disease can undoubtedly produce enough cortisol to maintain levels within the normal range.

For example, in 9 untreated patients studied by the author the 9 a.m.–10 a.m. plasma 11-hydroxycorticoid levels ranged from zero to  $17.9 \mu\text{g}/100 \text{ ml.}$ , and only four of these patients had levels below  $5.7 \mu\text{g}/100 \text{ ml.}$ , which is the lowest limit of the normal range at this time of day.

Addison's disease can be confirmed in an untreated patient within a few hours of admission to hospital if the rise in the plasma 11-hydroxycorticoid level five hours after a single intramuscular injection of 50 units of ACTH gel is used to measure the adrenal response to stimulation. The results of this

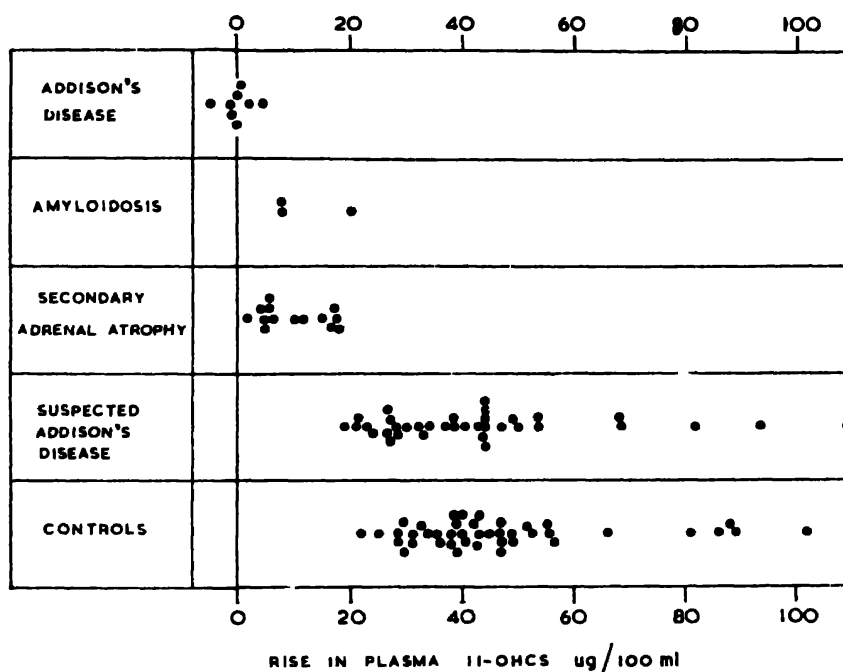


FIG. 5.5. Plasma 11-hydroxycorticoid response in 103 hospital patients five hours after a single intramuscular injection of 50 units of ACTH gel. (See text.)

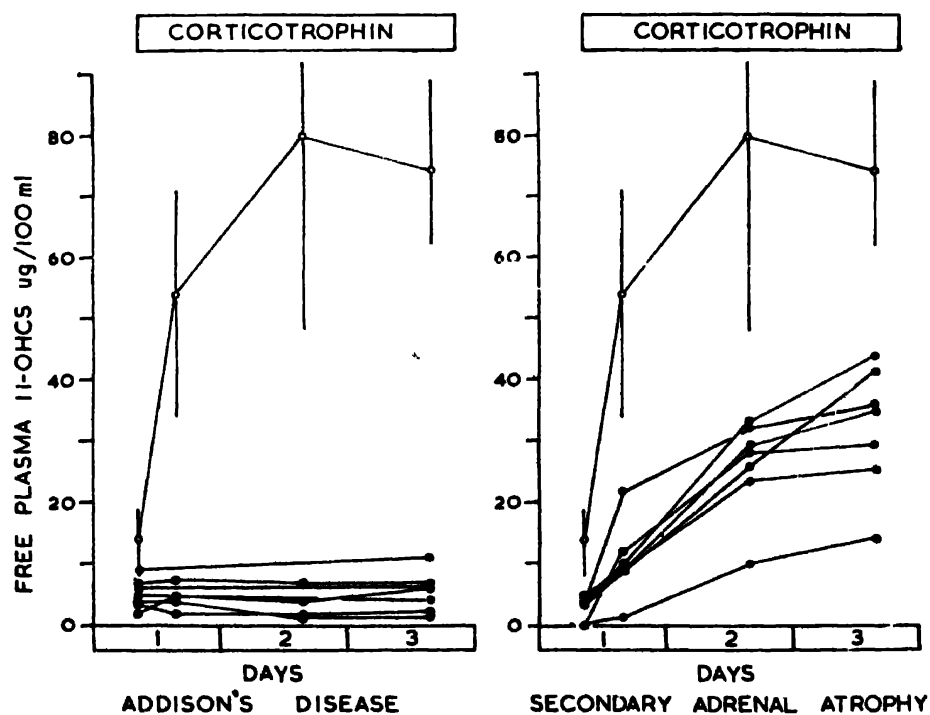


FIG. 5.6. Plasma 11-hydroxycorticoid response to prolonged ACTH stimulation in 7 patients with Addison's disease and 7 patients with secondary adrenal atrophy. The mean response in 11 normal subjects is shown by the open circles, the vertical lines representing the range of observations. (See text.)

test in 103 hospital patients are shown in Fig. 5.5. Eight patients with Addison's disease failed to show a significant rise, whilst two out of three patients with secondary amyloidosis involving their adrenals had subnormal responses. Addison's disease was excluded in 36 other patients in whom the diagnosis had been suspected on clinical grounds.

There are occasions, however, when it is necessary to confirm the diagnosis of Addison's disease in patients who have already been started on corticosteroid therapy. In these patients a negligible response to one injection of ACTH gel is not diagnostic of primary adrenal failure since it may be due to the secondary adrenal atrophy produced by the corticosteroids (Fig. 5.5). This difficulty can be overcome by prolonging the ACTH stimulation for several days. ACTH gel is given by deep intramuscular injection in a dose of 50 units twice a day for three days, and the plasma 11-hydroxycorticoid level measured before the first injection and five hours after each morning injection. There is no need to stop steroid treatment during the test but synthetic corticosteroids must be substituted for cortisol or cortisone at least 48 hours beforehand. Since the synthetic steroids do not fluoresce, the plasma 11-hydroxycorticoid levels during ACTH administration will be a measure of the patients' adrenal response. Patients with Addison's disease show no response to this test, but those with secondary adrenal atrophy show a significant rise in the plasma 11-hydroxycorticoid level by the second or third day (Fig. 5.6).

Urinary steroid estimations can also be used to determine the adrenal response to ACTH stimulation, but these are more time consuming and require the accurate collection of 24-hour urine samples.

**Treatment.** Most patients with Addison's disease can be maintained in good health on 37.5–50 mg of cortisone daily, given in divided doses three times a day. Additional salt is usually unnecessary if small doses of fludrocortisone are given to supplement the weak sodium-retaining properties of the cortisone. Alternatively, 50–75 mg of deoxycorticosterone trimethyl acetate can be given by intramuscular injection every three weeks. Fludrocortisone is a very potent mineralocorticoid and patients rarely require more than 0.05 mg daily, or 0.1 mg on alternate days, to keep them in sodium balance. As little as 0.2 mg daily can produce a profound potassium depletion with muscular weakness and a hypokalaemic alkalosis. A rising blood pressure and rapid gain in weight are signs of mineralocorticoid over-dosage.

### **Damage to the ACTH-releasing Mechanisms**

Disease of the hypothalamus or pituitary gland may damage the ACTH-releasing mechanisms. By far the commonest cause is destruction of the pituitary gland, either by tumour or by infarction following a severe post-partum haemorrhage. In the absence of ACTH the adrenal glands will atrophy and the output of glucocorticoids and androgens will fall to a very low level. The zona glomerulosa, which produces aldosterone, does not share in this atrophy so that aldosterone secretion is not normally affected. Complete destruction of the pituitary will result in a loss of all its trophic hormones, and the clinical picture of cortisol deficiency will be accompanied by signs of hypothyroidism and lack of gonadal function. Incomplete destruction, on

the other hand, may produce no overt signs of hypopituitarism but ACTH production may be sufficiently impaired to prevent the normal pituitary-adrenal response to stress. The measurement of plasma ACTH levels would provide valuable information about the integrity of the ACTH-releasing mechanisms, but the present bioassay methods are too laborious for routine clinical use. However, these mechanisms can be tested indirectly by studying adrenal function since the production of cortisol by the adrenal glands is extremely sensitive to changes in the level of circulating ACTH.

The investigation of adrenal function in patients with severe hypopituitarism will reveal a profound secondary adrenal atrophy. Morning plasma 11-hydroxycorticoid levels may be less than 6  $\mu\text{g}/100\text{ ml.}$ , and the response to ACTH stimulation will be markedly impaired (Figs. 5.5, 5.6). The urinary 17-oxosteroids are often reduced to 1–2 mg/24 hours, but the urinary 17-hydroxycorticoids and 11-hydroxycorticoids may fall within the lower limits of the normal range. They will, however, fail to rise normally after ACTH stimulation. Low plasma sodium concentrations are quite common in panhypopituitarism and are a sign of cortisol deficiency with consequent overhydration of the plasma. Water excretion tests are particularly dangerous in these patients as they may produce water intoxication resulting in confusion, convulsions and even coma.

Adrenal function tests are often normal in patients with incomplete pituitary destruction, or hypothalamic disorders, but metyrapone or vasopressin tests may reveal a diminished ability on the part of the pituitary to secrete ACTH. If either of these tests is abnormal it is reasonable to assume that the pituitary-adrenal response to stress will be impaired and to give hydrocortisone to these patients during operations and other stressful situations.

### **Corticosteroid Therapy**

Corticosteroids inhibit the pituitary secretion of ACTH and their prolonged administration results in secondary adrenal atrophy. Graber and his co-workers (1965) have recently studied plasma ACTH and cortisol levels in a number of patients and found that pituitary-adrenal recovery after corticosteroid therapy followed a definite pattern, requiring several months for completion. Initially, both plasma ACTH and cortisol levels were relatively low, a situation similar to that seen in hypopituitarism. After a month or two the ACTH levels rose to supernormal levels but there was a lag of several months in the recovery of normal adrenal responsiveness.

These observations are of great practical importance for it would appear that the period of impaired pituitary-adrenal function might be reduced to a month or two in most of these patients if the secondary adrenal atrophy were corrected by a course of ACTH gel injections before the steroids are stopped. The adrenal response to this prolonged ACTH stimulation can be followed by measuring plasma 11-hydroxycorticoid levels five hours after the morning injections. The steroids and ACTH may be discontinued together as soon as a near-normal response is obtained. Recovery of the ACTH-releasing mechanisms can then be determined by measuring morning plasma 11-hydroxycorticoid levels for two to three days after stopping treatment (Fig. 5.7). Inability to maintain these levels within the normal range is clear

## WEANING FROM STEROIDS

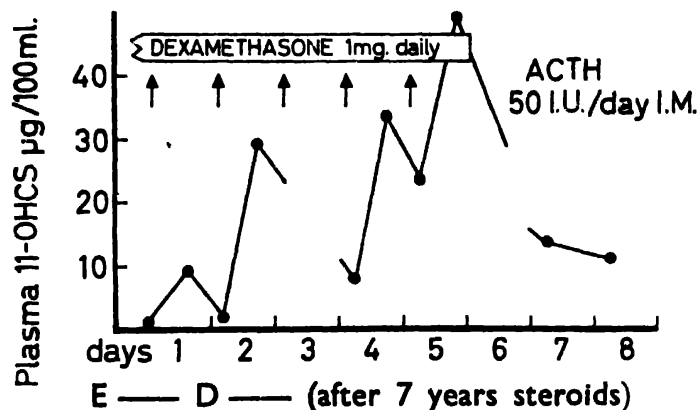


FIG. 5.7. Plasma 11-hydroxycorticoid levels after ACTH stimulation and successful steroid withdrawal in a patient who had been on corticosteroid therapy for seven years (from COPE, 1966).

evidence that the pituitary is unable to secrete enough endogenous ACTH of its own.

The number of patients with such severe impairment of their ACTH-releasing mechanisms is likely to be small and confined to those who have been on continuous treatment for more than three years. It is nevertheless important that they should be recognized before calamitous attempts to wean them from steroid therapy are made. In the majority of patients the pituitary-adrenal axis will be found to be intact, but the ability to secrete increased amounts of ACTH in response to stress is not tested by this procedure. Metyrapone tests have been used to determine this in the past but they are not entirely satisfactory for the reasons given earlier. It is possible that vasopressin tests may provide this information, but this has not been established yet.

### Acute Adrenal Insufficiency

The numbers at risk from acute adrenal insufficiency have increased enormously since the introduction of corticosteroids into clinical practice. Vomiting and diarrhoea are particularly dangerous in patients on corticosteroid therapy for the absorption of these drugs from the bowel may be impaired when either is present. Infection is by far the commonest precipitating factor but its presence may be masked by the anti-inflammatory action of the steroids.

Mental confusion or hysterical behaviour are often the first signs of an impending crisis and their significance may not be appreciated at the time. Abdominal pain, nausea, headache and drowsiness are common presenting symptoms. Unexplained fever is not uncommon and is usually accompanied by a rapid pulse. Hypotension is invariably present in Addison's disease during an Addisonian crisis, but a normal blood pressure does not exclude acute adrenal insufficiency when secondary adrenal atrophy is due to corticosteroid therapy.

Urinary steroid assays are of little value in the diagnosis of acute adrenal failure since treatment can rarely be withheld in order to collect adequate

urine samples. It is in these circumstances that random plasma 11-hydroxycorticoid estimations have been invaluable in confirming the diagnosis. Mattingly & Tyler (1965) found that the mean morning plasma 11-hydroxycorticoid level in 23 patients known to be suffering from acute adrenal insufficiency was only  $2.2 \mu\text{g}/100 \text{ ml.}$ , the range being  $0-5.4 \mu\text{g}/100 \text{ ml.}$

No levels as low as this were found in a group of 14 surgical patients and 23 medical patients who developed hypotension and were suspected of adrenal failure. None had a level below  $20 \mu\text{g}/100 \text{ ml.}$  and the levels were markedly elevated in the majority. Patients with levels above  $80 \mu\text{g}/100 \text{ ml.}$  rarely lived for more than 24 hours. A rise in blood pressure following intravenous hydrocortisone succinate does not necessarily confirm the diagnosis of hypoadrenalism.

There are many causes for a falling blood pressure in these sick patients and the rapid estimation of the plasma 11-hydroxycorticoid level will enable the diagnosis of adrenal failure to be excluded in most of them. Occasionally, levels up to  $10 \mu\text{g}/100 \text{ ml.}$  have been found in acutely ill patients with primary or secondary adrenal atrophy. Presumably their adrenal glands were able to produce a little cortisol but it was insufficient to meet the extra demands of stress. Intravenous hydrocortisone succinate should be given as soon as the diagnosis is suspected. Additional treatment with intravenous saline and intramuscular deoxycorticosterone acetate (DOCA) will be necessary to correct the sodium depletion in patients with primary adrenal failure.

## ADRENAL HYPERFUNCTION

### Cushing's Syndrome

Cushing's syndrome is rare in childhood and old age, and occurs mainly in women during the reproductive period of life. It is now generally accepted that the main clinical features of this disease are due to the prolonged excessive secretion of cortisol, and all of them have been accidentally reproduced at one time or another with high doses of cortisone or its synthetic analogues. The androgenic effects which are sometimes seen in this disorder are thought to be due to an accompanying increase in the output of adrenal androgens.

**Pathology.** Bilateral hyperplasia is the commonest abnormality which is found in the adrenal glands, being present in about 75 per cent of cases. An adenoma is present in about 15 per cent, and a carcinoma in the remaining 10 per cent. Adenomatosis occasionally develops in hyperplastic glands, and multiple discrete adenomas in both glands have been described (Chappell, 1963).

The most constant pituitary abnormality is hyalinization of the basophil cells with disappearance of the basophil granules. At one time it was thought that these changes might be the primary causative lesion but it is more likely that they are secondary to the high levels of circulating cortisol since they have also been found in cortisone-treated patients (Laqueur, 1951). Of much greater significance is the presence of a basophil or chromophobe adenoma of the pituitary gland in about half the patients with adrenal hyperplasia. These tumours are often too small to detect clinically but they may increase in size following bilateral adrenalectomy.

Cushing thought that the basic abnormality in this disease was the

excessive secretion of pituitary hormones, and there has been considerable support for this view in recent years. Nelson, Meakin & Thorn (1960) found extremely high plasma ACTH levels in patients with adrenal hyperplasia who developed pituitary tumours after adrenalectomy, and Liddle & Williams (1962) have shown that these high plasma ACTH levels after operation are not limited to patients with demonstrable pituitary tumours. Elevated plasma ACTH levels have also been reported in some untreated patients with adrenal hyperplasia (Ney *et al.*, 1963; Prunty *et al.*, 1963). It seems highly likely that the basic abnormality in this form of Cushing's syndrome is the excessive secretion of ACTH with a resulting over-production of cortisol by the adrenal glands. In these patients the normal diurnal rhythm of ACTH secretion is lost and the pituitary is not inhibited by the normal feed-back mechanism.

Adrenal hyperplasia is sometimes associated with a "non-endocrine" tumour. This may be a carcinoma of the breast, colon, gallbladder, ovary, pancreas, prostate gland, thymus or trachea, but is more commonly an oat-cell carcinoma of the bronchus. Many of these patients have very high cortisol secretion rates and gross adrenal hypertrophy is often found at autopsy. Curiously enough, these high cortisol secretion rates are not always associated with the typical features of severe Cushing's syndrome, and this diagnosis may not be suspected in life. Although this association was first described nearly 40 years ago (Brown, 1928), it is only recently that a rational explanation for it has been found. It would appear that the excessive stimulus to the adrenal glands in these patients is coming from the tumour and not from the pituitary gland. Meador and his colleagues (1962) found an ACTH-like substance in extracts of the plasma and tumour tissue from 5 patients with this syndrome, and their findings have been confirmed by other workers (Liddle *et al.*, 1963; Jarett, Lacy & Kipnis, 1964).

Adrenal tumours appear to function autonomously, and the inhibition of pituitary ACTH secretion by cortisol from the tumour results in atrophy of the other adrenal gland. Adrenal carcinomas sometimes secrete more than one hormone in excess and the clinical picture of Cushing's syndrome may be accompanied by virilism in women and feminization in men. High urinary oestrogen levels are found in some of these male patients (Gabrilove *et al.*, 1965).

**Clinical Features.** The severe case of Cushing's syndrome usually presents no difficulty in diagnosis, but adrenal hyperfunction is often present in patients with few signs and symptoms suggestive of classical Cushing's syndrome. There is no doubt that spontaneous remissions do occur in patients with adrenal hyperplasia, and the fluctuating course of this disease may extend over many years before the diagnosis is finally confirmed. Ross, Marshall-Jones & Friedman (1966), in a recent survey of 50 cases, have stressed the necessity for considering the possibility of Cushing's syndrome in any patient who presents with any of the known manifestations of this disease. An interesting computer analysis of the features of Cushing's syndrome has been recently undertaken by Nugent (1964). While obviously an indication of the way in which diagnostic problems will be tackled in the future, it remains at present too specialized an approach to be considered here (see Chapter 1, p 15).



TABLE 5.II

PERCENTAGE INCIDENCE OF MAIN FEATURES OF CUSHING'S SYNDROME  
IN 36 PATIENTS

	%		%
Obesity	97	Easy bruising	39
Hypertension	76	Edema	36
Plethoric facies	72	Dyspnoea on exertion	33
Menstrual disturbances	69	Purple striæ	25
Abnormal glucose tolerance test	68	Acne	22
Radiological osteoporosis	64	Enlarged pituitary fossa	17
Hirsutism	61	Pink striæ	8
Muscular weakness	44	Pigmentation	8
Glycosuria	42	Polycythæmia	3
Depression	39		

The percentage incidence of the main clinical features of Cushing's syndrome in 36 patients studied by the author is shown in Table 5.II. The series consisted of 28 females and 8 males and included 27 patients with adrenal hyperplasia, 5 with hyperplasia associated with a "non-endocrine" carcinoma, 2 with an adrenal adenoma, and 2 with an adrenal carcinoma. Cushing's syndrome was usually suspected because of the combination of obesity, plethoric facies, hypertension and an abnormal glucose tolerance curve, but the purple striæ which are supposedly diagnostic of this disorder were present in only 9 of these 36 patients.

**Laboratory Tests.** The diagnosis of Cushing's syndrome can only be established with certainty by demonstrating that cortisol production is

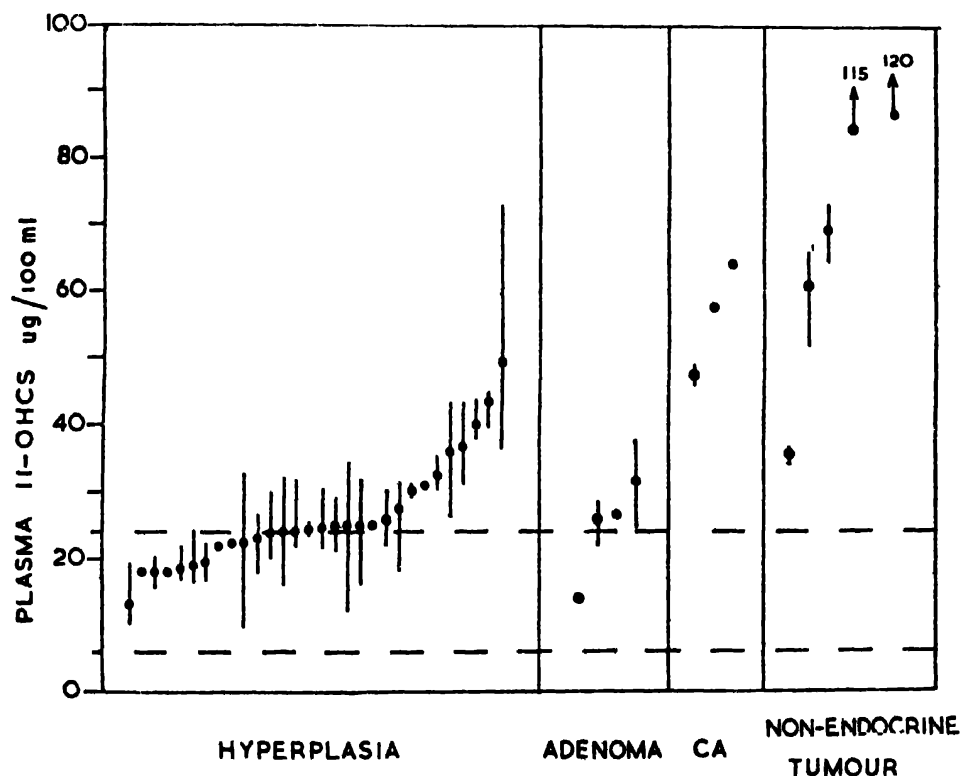


FIG. 5.8. Plasma 11-hydroxycorticoid levels between 9 a.m. and 10 a.m. in 42 patients with Cushing's syndrome. The day-to-day variation in some of these patients is shown by the vertical lines. The horizontal dashed lines show the normal range at this time of day.

increased above the upper limit of normal. Brooks *et al.* (1963) studied 25 patients with this disorder and concluded that the most reliable index of increased adrenocortical activity was the cortisol secretion rate, which was elevated in all 21 patients in whom it was measured. The next most useful measurement was the excretion of free urinary cortisol which was elevated in 17 out of 21 patients. By contrast, the 17-oxogenic steroid excretion was within the normal range in 11 of the 25 patients, and the plasma cortisol level was raised only in 9 of the 17 patients in whom it was measured. These findings are in general agreement with those of other workers (Cope &

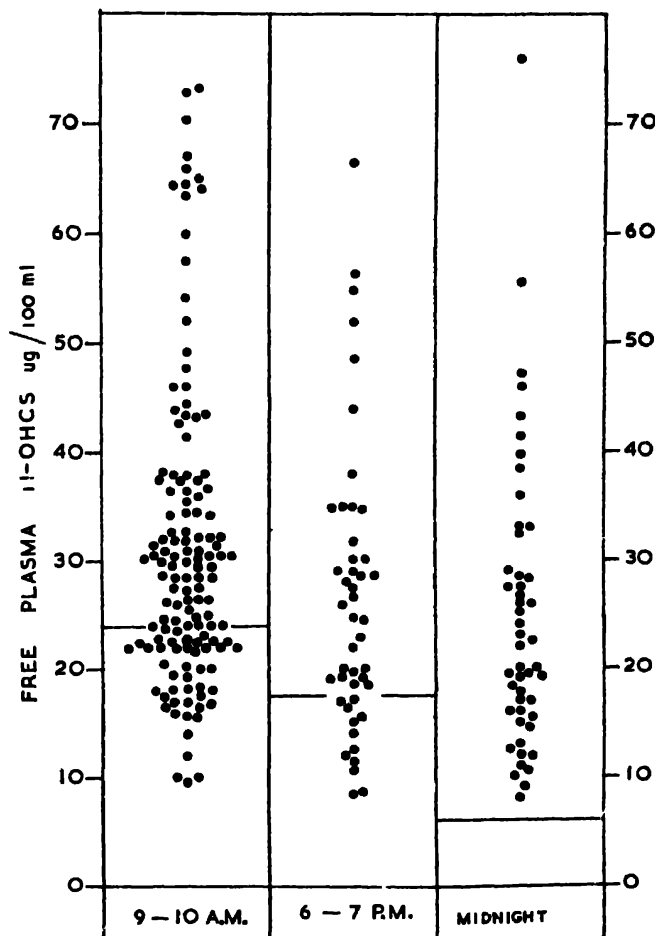


FIG. 5.9. Lack of normal diurnal rhythm in plasma 11-hydroxycorticoid levels of 42 patients with Cushing's syndrome. The upper limits of the normal ranges are shown by the horizontal lines (from COPE, 1966).

Pearson, 1965; Ross, Marshall-Jones & Friedman, 1966). Cope & Pearson (1965), for example, found elevated cortisol secretion rates in 30 patients with Cushing's syndrome, but in 7 of these the 17-oxogenic steroid excretion was less than 15 mg/24 hours.

The measurement of the cortisol secretion rate and free urinary cortisol are outside the scope of most hospital laboratories, but the diagnosis of Cushing's syndrome can be made with less precise measures of adrenocortical function provided that their limitations are borne in mind. Mattingly *et al.* (1964) found raised urinary 11-hydroxycorticoid levels, which ranged from 400 to 7,000  $\mu$ g/24 hours, in 20 patients with Cushing's syndrome. Elevated

levels have since been found in a further 16 patients studied by the author. Normal levels, on the other hand, have been found in a group of 33 patients with "simple" obesity.

Plasma 11-hydroxycorticoid levels have been less helpful in the diagnosis of Cushing's syndrome since in only about half the patients studied have the morning levels been consistently higher than the upper limit of the normal range at this time of day (Fig. 5.8). Midnight plasma 11-hydroxycorticoid levels are, however, of much greater diagnostic value for the normal diurnal rhythm of the plasma cortisol level is absent or much reduced in this disease (Fig. 5.9).

The estimation of urinary 17-oxogenic steroids or 17-hydroxycorticoids are the least helpful of all the methods in current use for the reasons given earlier, but they usually exceed 50 mg/24 hours in patients with adrenal carcinomas and in patients with hyperplasia associated with carcinomas in other organs. Normal levels do not exclude Cushing's syndrome and the converse is also true. Raised urinary 17-oxogenic steroids have been reported in patients with "simple" obesity, the levels falling to normal after weight reduction (Baird, 1963). The cortisol secretion rate has also been shown to be minimally increased in some patients with "simple" obesity, and this may lead to diagnostic difficulties in the border-line case. However, Gogate & Prunty (1963) found that there was no abnormality in urinary cortisol excretion in the 5 obese patients whom they studied. This was in direct contrast with their observations in Cushing's syndrome.

### Dexamethasone Suppression Tests

The administration of corticosteroids in large amounts to normal subjects results in a rapid suppression of pituitary ACTH secretion and a fall in adrenocortical activity to low levels. This suppression of adrenal activity does not occur in patients with adrenal tumours since these function independently of the pituitary, whilst patients with adrenal hyperplasia usually show some resistance to pituitary ACTH suppression. Oral dexamethasone suppression tests have therefore been used both to confirm the diagnosis of Cushing's syndrome when the basal data has been equivocal, and to differentiate between hyperplasia and tumour (Liddle, 1960; Slater *et al.*, 1962; Brooks *et al.*, 1963; Nugent, Nichols & Tyler, 1965). As yet there is no general agreement on the dose of dexamethasone to use, on the duration of the test, or on the best method of measuring the adrenal response to suppression. However, in most normal subjects the urinary 17-hydroxycorticoid excretion will fall to 4 mg/24 hours by the second day of dexamethasone administration if this drug is given in a dose of 0.5 mg every six hours.

Plasma 11-hydroxycorticoid estimations are a more convenient method of measuring adrenocortical function during dexamethasone administration since they are not dependent on the accurate collection of 24-hour urine specimens and the answer to the test can be obtained the same day. Blood samples are taken between 9 a.m. and 10 a.m. to avoid the normal diurnal variation in plasma cortisol levels, and plasma 11-hydroxycorticoid levels are measured before and after 48 hours of dexamethasone administration (Mattingly, 1963). The response to 2 mg of dexamethasone daily is shown in Fig. 5.10. It will be seen that the plasma 11-hydroxycorticoid level in 14 normal

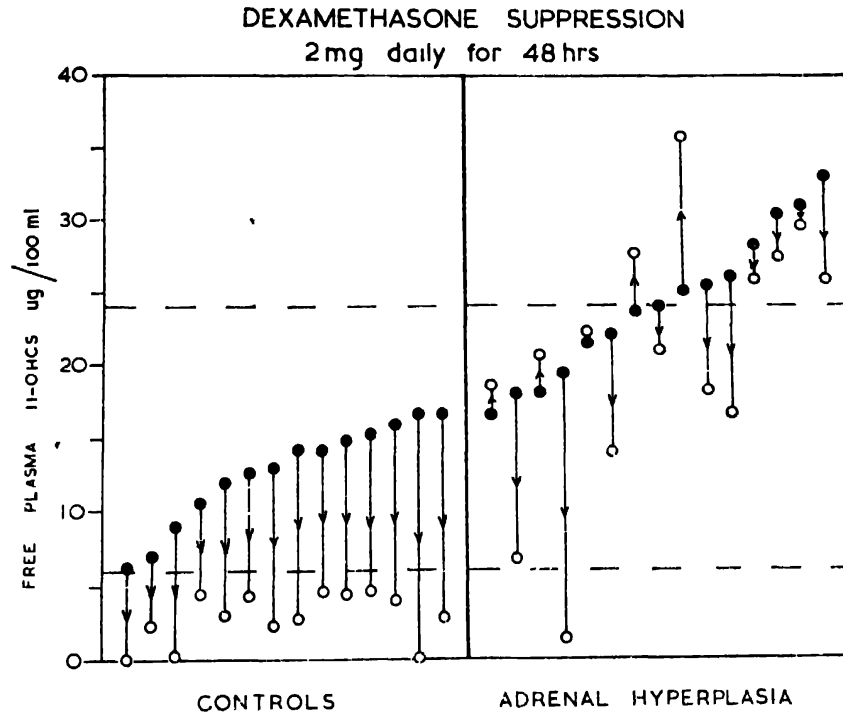


FIG. 5.10. Resistance of the pituitary-adrenal axis to suppression by dexamethasone in Cushing's syndrome due to adrenal hyperplasia. Closed circles—before dexamethasone. Open circles—48 hours on dexamethasone 2 mg daily. The horizontal dashed lines indicate the normal range between 9 a.m. and 10 a.m.

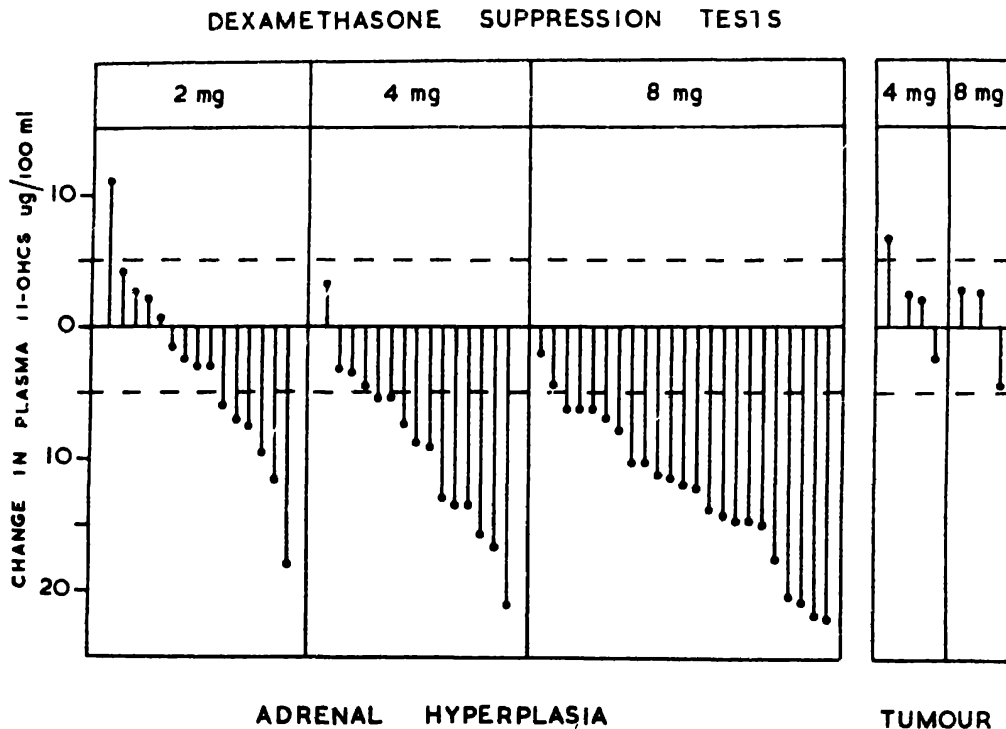


FIG. 5.11. The effect of increasing the daily dose of dexamethasone on morning plasma 11-hydroxycorticoid levels in Cushing's syndrome. The degree of suppression was measured by the fall in plasma 11-hydroxycorticoid levels from the baseline after 48 hours on each dose. The horizontal dashed lines show the 95 per cent fiducial limits for single estimations (from COPE, 1966).

subjects fell to less than 6  $\mu\text{g}/100\text{ ml.}$  at the end of 48 hours, but 13 out of 15 patients with adrenal hyperplasia failed to suppress on this dosage. The effect of increasing the dose of dexamethasone to 8 mg daily for 48 hours is shown in Fig. 5.11. Twenty-one out of 23 patients with adrenal hyperplasia showed significant suppression on this higher dose. No suppression was seen in 3 patients with adrenal tumours.

### Metirapone Tests

Metirapone tests have also been used to distinguish between hyperplasia and tumour (Liddle *et al.*, 1959) and are of particular value in those patients who fail to show any suppression on high doses of dexamethasone. No response is usually seen in patients with adrenal tumours, but exceptions have been reported (Martin & Hamman, 1966).

### ACTH Tests

ACTH tests are of very limited value in the diagnosis of this disorder. Excessive responses to ACTH, both in the plasma and urine, are not uncommon in Cushing's syndrome due to bilateral hyperplasia, but there is a considerable overlap with the results found in hospital patients suffering from other diseases. Occasionally an exaggerated response is seen in a healthy subject which is equal to that found in the most severe cases of Cushing's syndrome. Although adrenal tumours acquire the capacity to function autonomously they may still respond to exogenous ACTH so that ACTH tests cannot be relied upon to differentiate with certainty between tumour and hyperplasia.

### Non-endocrine Tumours

The presence of a "non-endocrine" tumour should be suspected in any patient with Cushing's syndrome who presents with a hypokalaemic alkalosis. This abnormality, however, is not confined to this group of patients with Cushing's syndrome (Christy & Laragh, 1961; Prunty *et al.*, 1963). Aldosterone secretion rates are not elevated in these patients, and it has been suggested that the high secretion rates of cortisol are responsible for their potassium depletion. Failure to suppress adrenal activity with high doses of dexamethasone is not uncommon, and there may be no response to metirapone.

**Treatment.** Removal of an adrenal tumour, or of both adrenals in cases of hyperplasia, leads to a remission of the disease. The recurrence rate following sub-total adrenalectomy for hyperplasia is of the order of 30 per cent, and this operation is done less frequently than in the past (Sprague *et al.*, 1961). Total adrenalectomy is a simpler procedure for the surgeon and guarantees a remission, provided that there is no appreciable mass of aberrant adrenocortical tissue. The main disadvantage of total adrenalectomy is the substitution of Addison's disease for Cushing's syndrome, but these patients can be kept in good health on adequate doses of cortisone and a mineralocorticoid.

Although the majority of patients with hyperplasia are treated by total adrenalectomy there is considerable doubt as to whether this is the most satisfactory treatment for this condition. Removal of the hyperplastic

glands does not correct the excessive secretion of ACTH by the pituitary gland which is now thought to be the fundamental disorder in these patients, and the high plasma ACTH levels found post-operatively suggest that the pituitary is stimulated to even greater activity when the plasma cortisol level is dropped to normal. The incidence of rapidly growing pituitary tumours after adrenal surgery is between 5 and 10 per cent, and one cannot escape the conclusion that their growth has been stimulated by this procedure.

An alternative approach to the problem of treating Cushing's syndrome due to adrenal hyperplasia is to attack the pituitary gland itself, and a number of procedures have been developed to produce partial or complete pituitary ablation in this disease. These include hypophysectomy, external irradiation and the implantation of radio-active material into the pituitary fossa. Total ablation may be necessary if a tumour is present, but panhypopituitarism is a heavy price to pay for a remission in children and young adults. Partial pituitary ablation sometimes follows conventional deep X-ray therapy to the pituitary gland, but only about half the patients treated in this way have had a remission of their disease (Dohan *et al.*, 1957; Soffer, Iannaccone & Gabrilove, 1961). The introduction of radio-active seeds into the pituitary fossa enables a larger dose to be delivered to the pituitary gland, but the seeds must be accurately placed to avoid damage to the surrounding structures, and the dose carefully calculated to avoid complete destruction of the pituitary tissue (Fraser & Joplin, 1961). Hartog and his colleagues (1965) have recently reported the results of partial pituitary ablation with radio-active gold or yttrium seeds in 20 patients with adrenal hyperplasia. Eight patients had a satisfactory response to their first implant and a partial response occurred in eight others. Complications arose mainly in the six patients with an obvious pituitary tumour. Three of these patients developed a cerebrospinal fluid leak and none had a permanent remission of their disease. These results are rather disappointing for it is in this particular group of patients that one is seeking an alternative to adrenalectomy.

The primary treatment of adrenal carcinoma should be surgical removal of the tumour whenever possible, but these tumours are very malignant and are frequently inoperable by the time the diagnosis is made. Bergenstal and his colleagues (1960) reported that the administration of the drug *o,p'*-DDD to patients with functioning adrenal cortical carcinomas resulted in a significant regression of measurable metastatic lesions, associated with a decrease in abnormal steroid excretion. This has now been confirmed by Hutter & Kayhoe (1966) in a series of 138 patients with adrenal cancer. *o,p'*-DDD is a derivative of the insecticide DDD and produces necrosis and atrophy of the adrenal cortex. The prolongation of life produced by this drug is only measurable in terms of months, but it does hold out hope that less toxic and more effective drugs may be found in the future to treat these otherwise hopeless cases.

If a less toxic drug than *o,p'*-DDD is developed it might even replace adrenalectomy in the treatment of patients with adrenal hyperplasia. Southren and his colleagues (1966) have already demonstrated that *o,p'*-DDD can produce a remission in such a patient, but the toxic effects of *o,p'*-DDD on the gastrointestinal tract and central nervous system are likely to severely restrict the use of this particular compound.

### Adrenal Virilism

Virilism is defined as the development of masculine physical and mental characteristics in the female, and is caused by the excessive secretion of androgens from the adrenal glands or ovaries. Physical signs include hirsutism, acne, clitoral enlargement, deepening of the voice and irregular or absent menstruation. Hirsutism, or excessive hairiness, is usually the first sign of androgen excess in the adult female and is particularly noticeable on the face and abdomen. In mild cases it may not be accompanied by any other signs of virilism. The over-production of androgens by the adrenal glands or testes in men may pass unnoticed, but in boys it will result in abnormally rapid growth and precocious puberty. Congenital adrenal hyperplasia is the commonest cause of virilism in infancy and childhood and the term "adrenogenital syndrome" is usually reserved for this disorder. Tumours of the gonads or adrenal glands are rare causes of virilism or precocious puberty, but should always be considered in the differential diagnosis of these conditions.

### Congenital Adrenal Hyperplasia

Congenital adrenal hyperplasia is a relatively uncommon condition in which the synthesis of cortisol is impaired by a wide variety of partial or complete enzyme deficiencies. These defects are probably inherited as recessive traits which can express themselves only in the homozygous offspring. The impaired production of cortisol results in plasma cortisol levels which are too low to inhibit the secretion of ACTH by the normal feed-back mechanism. As a result, these patients secrete far greater amounts of ACTH than normal and this leads to adrenal hyperplasia and to the over-

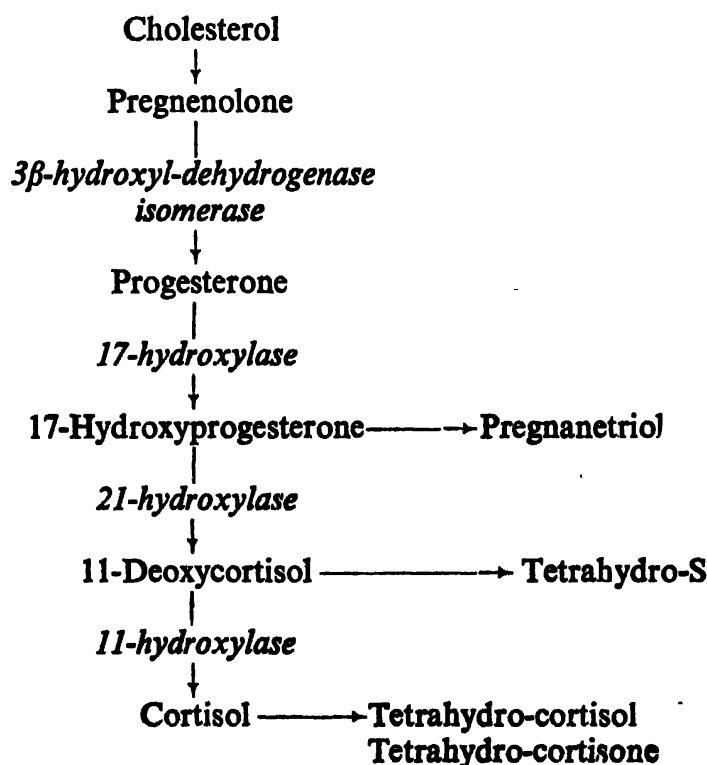


FIG. 5.12. Synthesis of Cortisol.

production of cortisol precursors and adrenal androgens. This excessive stimulation of the adrenal glands usually overcomes the block in cortisol synthesis, but there may be no adrenal reserve to meet the extra demands of stress. This condition is usually recognized within the first three or four years of life, but less severe forms may escape detection until much later. Carswell, Logan & Allison (1966) have recently confirmed the diagnosis in a man of 58 who presented with Addisonian pigmentation and infertility, and they suggested that the urinary 17-oxosteroids should be estimated in all cases of male infertility.

The synthesis of cortisol starts with cholesterol which loses most of its side chain at carbon atom 17 to form pregnenolone (Fig. 5.12). This, in turn, is converted into progesterone by the action of two enzymes. One of these,  $3\beta$ -hydroxyldehydrogenase, changes the hydroxyl group on carbon atom 3 to a ketone group. A rare cause of congenital adrenal hyperplasia is a deficiency of this enzyme, and Bongiovanni (1962) has reported three children in whom he considered this to be the major defect.

Progesterone is converted into cortisol by the successive action of three separate enzymes which insert hydroxyl groups on carbon atoms 17, 21 and 11. Failure of 21-hydroxylation is the commonest defect in congenital adrenal hyperplasia and accounts for about 90 per cent of cases. A block at this level results in an increased production of 17-hydroxyprogesterone and its metabolites are excreted in abnormal amounts in the urine. The estimation of one of these metabolites, pregnanetriol, is of great value in establishing the diagnosis of this particular defect. Normally pregnanetriol excretion does not exceed 2.5 mg/24 hours in adults and is usually less than 0.1 mg/24 hours before puberty (Visser, 1966). Pregnanetriol is a 17-hydroxycorticoid and the urinary 17-hydroxycorticoids may also be elevated in these patients. The urinary 17-oxosteroids are above the upper limit of normal for the age of the patient, reflecting the increased output of adrenal androgens.

A much rarer defect in congenital adrenal hyperplasia is a block in the final stage of cortisol synthesis. These patients have a deficiency of the enzyme  $11\beta$ -hydroxylase which inserts the final hydroxyl grouping on carbon atom 11. The metabolic abnormalities in this form of the adreno-genital syndrome are similar to those produced by metyrapone, and large amounts of 11-deoxycortisol (compound S) are produced instead of cortisol. The diagnostic feature of this defect is the presence of increased amounts of tetrahydro S in the urine, but few laboratories are equipped at present to carry out this estimation. However, tetrahydro S is a 17-hydroxycorticoid and the urinary 17-hydroxycorticoids and 17-oxosteroids are usually elevated in these patients. This disorder was first recognized by Eberlein & Bongiovanni (1955) in a child with hypertension. The production of both corticosterone and aldosterone is impaired in some patients with congenital adrenal hyperplasia and they suggested that the hypertension in their patient was due to the production of 11-deoxycorticosterone (DOC) instead of corticosterone.

### **Salt-losing Adreno-genital Syndrome**

About a third of the cases of congenital adrenal hyperplasia lose excessive amounts of sodium in their urine. It has been shown that aldosterone production is impaired in these patients (Bryan, Kliman & Bartter, 1962;



Kowarski *et al.*, 1965; New, Miller & Peterson, 1966), but there is some uncertainty as to whether aldosterone deficiency alone is responsible for their salt-losing state. That the salt loss is being actively promoted by some abnormal secretion of the adrenal glands is suggested by the increased salt loss which has been reported in some of these children when they are given ACTH. At one time it was thought that they might be secreting a specific salt-losing hormone but this has not been confirmed. An alternative explanation has been suggested recently by Kowarski *et al.* (1965). They found that aldosterone secretion rates were elevated in the non-salt-losing cases but were below the normal range in the more severe cases of the salt-losing form. Their explanation of these findings is based on the fact that an increased production of various cortisol precursors is a constant feature of congenital adrenal hyperplasia, and that at least two of these precursors, namely progesterone and 17-hydroxyprogesterone, are known to be aldosterone antagonists. They suggested that the salt-losing phenomenon in this disease was due to the combination of the over-production of aldosterone antagonists and a decreased ability to synthesize aldosterone, whilst in the non-salt-losing form the enzyme deficiency was less complete and these patients could secrete enough aldosterone to overcome the salt-losing effect of the aldosterone antagonists.

George, Saucier & Bartter (1965), on the other hand, found no significant increase in urinary sodium excretion when they gave ACTH to six patients with the salt-losing form of this disease, and concluded that the impaired aldosterone production in these patients was sufficient to account for their inability to conserve sodium. They confirmed the sodium-losing effect of progesterone but they pointed out that this was not striking, even in doses of 200–400 mg a day.

**Diagnosis in Infancy.** A severe impairment of cortisol synthesis is incompatible with survival, and an affected infant may die in the first week or two of life unless the condition is recognized early. Congenital adrenal hyperplasia is a familial disorder so that a history of the disease in a sibling should not be overlooked.

Some degree of virilism is always present in female infants, with enlargement of the clitoris and partial or complete fusion of the labia. It is not possible to distinguish between female pseudo-hermaphroditism and a boy with hypospadias on external inspection alone, but the genetic sex can be settled quickly by examining the buccal cells or polymorphonuclear leucocytes for chromatin-positive material. In the male infant the penis is normal at birth and the only clinical clue which may be present is pigmentation associated with the high levels of circulating ACTH. Severely affected infants will vomit repeatedly and fail to thrive, and the biochemical abnormalities in their plasma will be identical to those found in Addison's disease.

The collection of accurate 24-hour urine collections in these infants is very difficult, but the diagnosis of this disorder has been made much easier by the introduction of the method described by Morris (1959) for the differential estimation of the 17-oxogenic steroids in a casual urine sample. This method separates the 17-oxogenic steroids into an 11-deoxy group, which includes pregnanetriol, and an 11-oxygenated group consisting mainly of cortisol metabolites. The ratio (R) of the 11-deoxy group to the

11-oxygenated group is less than 0.5 in normal children, irrespective of age, but is invariably greater than this in children with congenital adrenal hyperplasia (Hill, 1960). R is increased in this disorder because there is an over-production of steroids lacking the final oxygen atom on carbon atom 11, and a reduced output of 11-oxygenated steroids. Barratt and his colleagues (1964), using a modification of this method, have confirmed the value of this ratio in the rapid diagnosis of this condition.

Plasma 11-hydroxycorticoid estimations are of no value in the diagnosis of cortisol deficiency in these children and can be very misleading. Elevated plasma 11-hydroxycorticoid levels have been found in a number of children with 21-hydroxylase deficiency (Steendijk, 1966, personal communication), and this may be due to the presence of 21-deoxycortisol in their plasma Wieland *et al.*, 1965).

**Differential Diagnosis.** Nine out of ten patients with congenital adrenal hyperplasia have a 21-hydroxylation defect and excrete increased amounts of 17-oxosteroids and pregnanetriol in the urine. However, these findings are not sufficient to establish this diagnosis since many patients with adrenal tumours also excrete excessive amounts of these steroids. Pregnanetriol excretion is normal in patients with gonadal tumours but the 17-oxosteroids are often raised. Dexamethasone suppression tests are of considerable value in differentiating hyperplasia from tumour, and the effect of inhibiting pituitary ACTH secretion can be followed by measuring the fall in the urinary 17-oxosteroids. Some initial resistance to dexamethasone suppression is not uncommon in congenital adrenal hyperplasia, and it may be necessary to give 8 mg daily for several days in order to be sure that suppression has occurred.

**Treatment.** Cortisone inhibits the pituitary secretion of ACTH and suppresses androgen production by the adrenal glands. The aim is to give the minimum dose necessary to keep the ratio (R) below 0.5 or the 17-oxosteroid excretion below 5 mg/24 hours. It is sometimes difficult to establish satisfactory suppression in children with oral cortisone, and there is a place for parenteral corticosteroid therapy in these patients. An intramuscular injection of 100 mg of cortisone or hydrocortisone acetate may produce adequate suppression for three or four days, whilst satisfactory suppression for as long as three weeks has followed a single injection of 100 mg of prednisolone trimethyl acetate. Children with the salt-losing form of the disease will need extra salt and 0.1–0.2 mg of 9 $\alpha$ -fluorohydrocortisone a day to keep them in sodium balance.

### Postpubertal Hirsutism

Some degree of hirsutism is not uncommon in women during the reproductive period of life. The usual age of onset is soon after puberty, and in severe cases there may be some frontal recession of the scalp hair. This excessive hair growth is often associated with acne, but other signs of virilism are uncommon.

Postpubertal hirsutism is probably due to a moderate increase in the output of androgens from the adrenal glands, the ovaries, or both. On rare occasions it may be due to phenytoin. Ovarian causes include bilateral

polycystic ovaries (Stein-Leventhal syndrome), solitary ovarian cysts, hilar cell hyperplasia, and some solid ovarian tumours. An obvious ovarian cause cannot be found, however, in the greater proportion of these hirsute women, and suspicion has not unnaturally fallen on the adrenal glands. Elevated 17-oxosteroids have been found in some of these patients, whilst others have shown an abnormal response to ACTH. The ratio of 17-oxogenic steroids to 17-oxosteroids after ACTH stimulation is normally greater than 2, but is often less than this in women with postpubertal hirsutism (Prunty, Brooks & Mattingly, 1958).

It now seems likely that a number of these hirsute women are suffering from a mild form of the adreno-genital syndrome, appearing for the first time after puberty. Brooks and his co-workers (1960) investigated three young women with fairly severe hirsutism and found abnormalities in their urine which were identical with those found in prepubertal patients with a 21-hydroxylase deficiency. In one patient the urinary pregnanetriol was only slightly raised at 3.7 mg per day, but rose to 21 mg per day after ACTH stimulation. Two of these women had severe acne and definite clitoral enlargement.

Other mild enzyme deficiencies may be present in patients with so-called "idiopathic" hirsutism and Gabrilove, Sharma & Dorfman (1965) have recently reported the presence of a partial 11 $\beta$ -hydroxylase deficiency in two young women. Their diagnosis was based on the finding of excessive amounts of tetrahydro S in the urine.

The nature of the androgen secreted in excess by these hirsute women is not known, but the suspicion is growing that it may be testosterone since elevated plasma and urinary testosterone levels have been found in a number of these patients. Nichols, Nugent & Tyler (1966) found that the urinary excretion of testosterone glucuronide was increased in 11 women with "idiopathic" hirsutism and fell to normal or subnormal levels on prolonged corticosteroid therapy. Four of these 11 patients had normal urinary 17-oxosteroids before treatment.

Since small but significant increases in testosterone secretion have been demonstrated in hirsute women with normal urinary 17-oxosteroids it is clear that the latter estimations do not provide a satisfactory measure of androgen production in these patients. The term "idiopathic" hirsutism has little meaning, since the majority of these patients are inadequately investigated at the present time.

**Treatment.** Prolonged corticosteroid therapy has been given an extensive trial in women with postpubertal hirsutism (de Mowbray *et al.*, 1959; Mattingly, Mills & Prunty, 1960). The acne is often improved by this treatment but there is rarely any significant diminution in hair growth, although the hair may become softer and finer. The effects are not sufficiently beneficial to justify its use routinely. Sometimes a woman is very distressed by her hirsutism and in these cases a trial of corticosteroid therapy is worthwhile. As little as 7 mg of prednisolone acetate daily for several months may produce a marked improvement in the mental state without any significant effect on hair growth. It is possible that the psychological symptoms are the result of androgen excess, for they not infrequently return when treatment is stopped.

## ALDOSTERONISM

### Primary Aldosteronism (Conn's Syndrome)

Primary aldosteronism (or hyperaldosteronism) is defined as a condition in which the prolonged excessive secretion of aldosterone is due to a primary disturbance in the adrenal glands. It is not associated with an increased production of cortisol. This condition was first recognized by Conn (1955) in a woman who presented with mild hypertension, episodic muscular weakness, nocturnal polyuria and polydipsia. Investigations revealed a hypokalaemic alkalosis which was associated with a decreased ability on the part of the kidneys to conserve potassium. Excessive amounts of aldosterone were found in the urine but the urinary 17-oxosteroids and 17-hydroxy-corticoids were normal. The patient became normotensive, and all the metabolic abnormalities disappeared, after removal of the right adrenal gland which contained a benign cortical adenoma.

This condition is not uncommon, for several hundred cases have been reported in the literature already (Conn, 1963). The commonest adrenal abnormality has been a solitary benign adenoma, but in about 15 per cent of cases more than one adenoma has been found. Occasionally this syndrome is associated with an adrenal carcinoma. Urinary aldosterone levels have been raised in about 90 per cent of these patients, and an increased aldosterone secretion rate has been found in most of the patients in whom it has been measured.

Glucose tolerance is impaired in about half the patients with Conn's syndrome and can be restored to normal by removal of the tumour tissue, or by potassium loading. Conn (1965) has shown that there is a delayed rise in plasma insulin levels following a glucose load in these patients which is similar to that seen in "maturity-onset diabetes". He has suggested that this delay is due to the effect of potassium depletion on the beta cells of the pancreas.

### Normokalaemic Primary Aldosteronism

There is a widespread belief that primary aldosteronism can be distinguished from "essential" hypertension by the presence of a persistent hypokalaemia. This view is no longer tenable since the demonstration by Conn and his colleagues (1965) of two aldosterone-secreting adenomas in a man of 39 who presented with "essential" hypertension and persistently normal serum potassium levels. The pre-operative diagnosis in this case was based on evidence of aldosterone overproduction and suppressed plasma renin activity, a combination which these workers have found only in patients with primary aldosteronism.

It would appear that hypokalaemia and severe potassium depletion are late and inconstant manifestations of primary aldosteronism and that many hypertensive patients without hypokalaemia could be cured by adrenal surgery. Just how many is difficult to predict with any certainty, but the incidence of so-called "non-functioning" adrenal cortical adenomas found at autopsy in hypertensive patients is about 20 per cent, compared to an incidence of 1.8 per cent in normotensive controls (Sherwin, 1964).

Conn has calculated that there may be three million hypertensive patients

with undiagnosed primary aldosteronism in the U.S.A. alone, and that about half of these may have an impairment of glucose tolerance indistinguishable from that seen in "maturity-onset diabetes". The only way to screen this enormous population for primary aldosteronism is to determine which ones have suppressed levels of plasma renin activity, but the methods available at present are too complex for mass screening. It is hoped that an immunoassay for plasma renin will be developed soon to enable this to be started. The ætiology and diagnosis of aldosteronism, with special reference to the renin-angiotensin system, are discussed in Chapter 10.

### Secondary Aldosteronism

Secondary aldosteronism is defined as a condition in which the excessive production of aldosterone has been stimulated by extra-adrenal factors. Bilateral adrenal hyperplasia may develop after prolonged stimulation. The commonest form of secondary aldosteronism, which is not normally accompanied by hypertension, is that associated with œdematous states such as the nephrotic syndrome, cirrhosis of the liver, and congestive cardiac failure. Hypokalaemia may be precipitated by diuretic therapy, but potassium depletion can usually be avoided by restricting the intake of sodium and by giving potassium supplements and aldosterone antagonists.

Secondary aldosteronism is often present in patients with malignant hypertension, or with hypertension associated with renal artery stenosis. Hypokalaemia is not uncommon and the clinical picture may suggest primary aldosteronism. There is some evidence that angiotensin II is the common stimulus in these patients, and plasma renin levels have been elevated in some of them.

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## CHAPTER 6

# IRON METABOLISM

by

ROGER WILLIAMS

RECENT studies in iron metabolism have led to a clearer picture of the processes concerned in the maintenance of normal stores and the regulation of iron absorption. Advances have also been made in our understanding of the various syndromes of iron deficiency and overload.

### NORMAL IRON STORES

The majority (2.1–2.5 g) of the total body iron (3–4 g) is present as circulating hæmoglobin. Muscle myoglobin contains approximately 130 mg (Bothwell & Finch, 1962). A further 115 mg is present in the labile pool and marrow erythrons (Pollycove & Mortimer, 1961). Very much smaller amounts are present in the plasma iron pool and in the various iron containing enzymes. The remainder constitutes the stores.

### Storage Compounds

Iron is stored in two distinct forms known as ferritin and hæmosiderin which were originally separated on the basis of differing water solubility.

**Ferritin** is water soluble. Individual molecules are too small to be visible by light microscopy but when present in tissue in high concentration may give a diffuse blue haze with Perl's technique. Each molecule consists of a shell, composed of the protein apoferritin, which surround micelles (colloidal complexes) of a ferric hydroxide and phosphate. The external diameter of the molecule in its native state is about 122 Å and that of the electron dense core about 74 Å (Harrison, 1964). The size of the central component is limited by the space available within the shell and the maximum iron content is 26 per cent of the molecular weight (for iron rich ferritin 800,000–860,000). On electron microscopy the core often appears to be composed of subunits and a view in which four subunits can be detected (tetrad form) is particularly characteristic. Other images are visible and various structures have been proposed such as four units at the vertices of a square or six units at the vertices of an octahedron (Muir, 1960).

Harrison (1964) has suggested that the structure of the micelles is determined by and is complementary to that of the apoferritin shell. She has shown that horse spleen apoferritin is made up of 20 subunits each composed of a single peptide chain situated at the 20 vertices of a pentagonal dodecahedron. If the micelles filled all the available space inside their shape would approximate to that of a solid icosahedron. X-ray diffraction studies suggest that the micelles are indeed polyhedral although their exact shape has not yet been determined.

The first stage in the formation of ferritin is the synthesis of apoferritin this occurs when ferrous iron enters the cell. Subsequently ferrous and phosphate ions pass through the spaces between the apoferritin subunits. Oxidation to ferric iron and the formation of micelles occurs once within the shell (Harrison, 1964).

Hæmosiderin is insoluble in water and can be visualized as golden-yellow granules in tissue sections which stain blue with Perl's technique. The iron in the granules is present as a ferric hydroxide-phosphate complex and the concentration is higher than in ferritin with a maximum of 45 per cent. Electron microscopy shows a variable structure. Some granules appear to be composed almost entirely of ferritin. In others there are closely aggregated electron dense particles but with none of the characteristic appearances of ferritin (Sturgeon & Shoden, 1964).

This variation in electron micrograph appearances is not surprising since there is increasing evidence that hæmosiderin is formed from ferritin. Ferritin, as a homologous protein, can stay freely dispersed in the cytoplasm of the cell until denatured by oxidizing agents. High concentrations of ferritin probably inhibit the activity of catalase and other protective enzymes so that  $H_2O_2$ , accumulating in the cell by auto-oxidation of flavoproteins, can readily oxidize the ferritin. The denatured molecules are then sequestered in vacuolar formations and granules. These structures may be visible with Perl's stain and biochemically this stage marks the transition from ferritin to hæmosiderin, although the characteristic structure of the ferritin micelles will still be visible on electron microscopy. Further degradation of the apoferritin matrix and aggregation of the micelles, resulting from the attack of proteolytic enzymes, leads to the final amorphous non-ferritinic structure of the mature hæmosiderin granule. (Matioli & Baker, 1963; Sturgeon & Shoden, 1964.)

The capacity of the cell to store ferritin is therefore limited whilst an almost unlimited amount can be stored as hæmosiderin. In control subjects about 65 per cent of the total stores of liver and bone marrow is present as ferritin. As the amount stored becomes in excess of normal there is a progressive rise in the proportion present as hæmosiderin (Morgan & Walters, 1963).

The iron in both forms of storage compound can be rapidly released when the need arises. Liberation of ferrous iron from ferritin can be easily achieved *in vitro* by the use of reducing agents. *In vivo* the xanthine oxidase system may be involved, the reduction of ferric to ferrous iron occurring in association with the oxidation of xanthine and hypoxanthine to uric acid. The marked decrease in hepatic ferritin content six-ten days after birth is closely related to an increase in hepatic xanthine oxidase activity. (Mazur & Carleton, 1965.) Powell & Emmerson (1966) have recently shown that the xanthine oxidase inhibitor, 4-hydroxypyrazolo (3, 4-d) pyrimidine (allopurinol), introduced for the treatment of gout, significantly increases the hepatic iron content of rats when added to their ordinary diet. The development of secondary hæmochromatosis in a patient with xanthinuria and a striking decrease of hepatic xanthine oxidase activity has also been reported (Ayvazian, 1964). Iron is probably also released from hæmosiderin in the ferrous form but less is known about the steps involved.

TABLE 6.I  
RECENTLY REPORTED VALUES FOR THE IRON CONTENT OF NORMAL ADULT LIVER

<i>Reference</i>	<i>Source</i>	<i>Sex</i>	<i>No. of Cases</i>	<i>Hepatic Iron† Concentration (mg/g wet weight)</i>	<i>Mean Total‡ Hepatic Iron Content (g)</i>
Morgan & Walters (1963)	Autopsies of traumatic deaths	M. F.	18 3	0.25 ± 0.03	0.39
Weinfeld (1965)	Surgical biopsies from uncomplicated gastric ulcer or gall bladder disease	M. F. (post-menopause) F. (menstruating)	29 11 13	0.24 ± 0.02 0.16 ± 0.03 0.09 ± 0.02	0.36 0.24 0.13
Powell (1966)	Autopsies of unexpected deaths	M. F.	68 32	0.26 ± .03 0.15 ± .02	0.47 0.23

† Mean values ± standard error.

‡ Assuming normal wet weight of 1,500 g and 30 per cent dry weight.

### Normal Total Body Iron

The only method for determining the total iron stores in man during life is by repeated venesections and estimation of the amount of iron mobilized and used for hæmoglobin synthesis. Pritchard & Mason (1964) found mean values of 819 mg in males and 254 mg in normal non-anæmic females. These figures are in agreement with separate estimates of the iron present in the major storage sites, liver, spleen and marrow, determined *in vivo* or at autopsy.

### Liver

The results of three recent studies in which iron concentrations were estimated chemically either in autopsies of unexpected deaths or in surgical liver biopsies show remarkably close agreement (Table 6.I). Powell (1966) was able to assess previous alcohol consumption by questioning close relatives and showed that the chemical iron concentration was lower and the Perl's reaction negative in 74 per cent of those who had taken alcohol in moderation (Table 6.II). Stainable iron was absent in only 27 per cent of those with

TABLE 6.I

THE RELATION BETWEEN HEPATIC HÆMOSIDERIN DEPOSITION AS JUDGED BY PERL'S STAIN AND IRON CONTENT MEASURED CHEMICALLY IN 100 PATIENTS. IN 65 PATIENTS IT WAS POSSIBLE TO ASSESS THE PREVIOUS ALCOHOL CONSUMPTION. HEAVY ALCOHOL CONSUMPTION WAS DEFINED AS MORE THAN 500 g ETHYL ALCOHOL PER WEEK (FROM DATA OF POWELL, 1966)

Hæmosiderin Grade†	Iron Concentration (mg/g wet weight)‡	Total Hepatic Iron (g)‡	No. of Cases with Light                  Heavy Alcohol Consumption	
0	0.14 ± .01	0.24 ± .02	37 (74%)	4 (27%)
1	0.27 ± .06	0.52 ± .06	11 (22%)	4 (27%)
2	0.65 ± .08	1.14 ± .15	2 (4%)	7 (47%)

† Graded according to criteria of Scheuer, Williams & Muir (1962).

‡ Mean values ± standard error.

a previously heavy alcohol consumption and in 47 per cent of the subjects in this group there was quite marked siderosis. Although the alcohol consumption had been high histological examination showed no evidence of liver damage.

MacDonald & Pechet (1965a) reported a much higher normal range of chemical iron concentration with a positive Perl's reaction in 53–80 per cent of patients in various autopsy series from different parts of the world. They included, however, patients with liver damage and other conditions known to affect iron storage. Such hospital control series are inevitably highly selective. Even cases of accidental death are not necessarily random. In the series of Powell (1966) 38 per cent of those with a heavy alcohol consumption committed suicide whereas only 10 per cent of the others died in this way.

### Spleen and Marrow

The concentration of iron in the spleen is less than that in the liver and in one autopsy series of accidental deaths the mean total content was 22 mg with a range of 10–57 mg (Morgan & Walters, 1963). Bone marrow is a more important site of storage. Gale, Torrance & Bothwell (1963) devised a method based on isotope dilution for determining total marrow stores *in vivo* and found average values of 288 mg in males and 99 mg in females.

Significant correlations have been reported between the marrow stores estimated either chemically or histochemically and those present in the liver. Weinfeld (1965), however, found the correlation far from absolute with an appreciable scatter of individual values. This is of some importance clinically because the histochemical grading of marrow iron has been regarded as one of the best methods available for assessing the body stores.

### Serum Iron and Total Iron-binding Capacity

Measurements of serum iron levels in normal subjects have shown a wide range (60–160  $\mu\text{g}/100\text{ ml.}$ ). There is a marked diurnal variation with the lowest level in the morning and also considerable variation from day to day

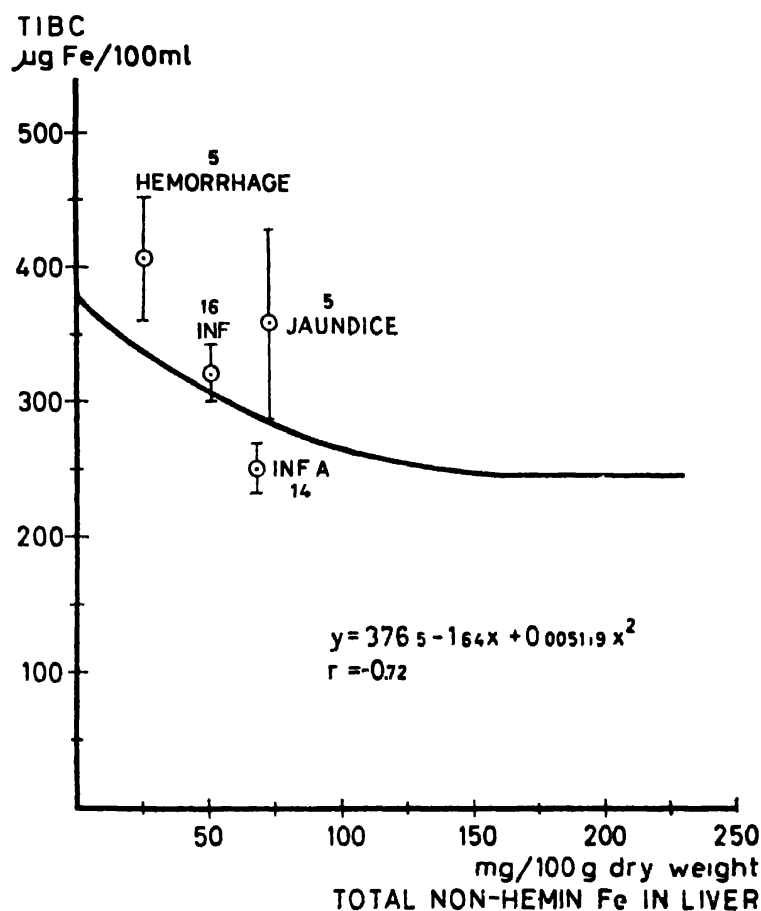


FIG. 6.1. Relation between the total iron-binding capacity and iron concentration in liver biopsy specimens for pathological groups compared with regression line for the basal group. The circles with the dot denote the mean deviation of the observed TIBC values from the basal curve. Vertical lines denote  $\pm$  twice the standard error of the mean deviation (from Weinfeld, 1965).

in the individual subject. In women the serum iron also varies during the menstrual cycle. A striking reduction occurs two–three days before the onset of bleeding and continues for two–three days. The mechanism is probably hormonal (Zilva & Patston, 1966). The serum iron is reduced when iron stores are exhausted and is often higher in iron overload but between these extremes shows little correlation with the stores (Weinfeld, 1965; Bainton & Finch, 1964).

The serum total iron-binding capacity (TIBC) is closely related to the size of the stores. The inverse correlation is curvilinear (Fig. 1), the changes in TIBC being greater when stores are low. The same relationship is found in disease states although here the level may be affected independently by other factors particularly disturbances in protein synthesis as in severe liver disease, infection or malnutrition. Current colorimetric or radioisotopic methods for measuring the TIBC do not take account of the occasional presence of forms of iron in the serum such as ferritin which are not bound to transferrin. This may be the explanation for the finding of lower TIBC values in some cases of liver disease when an immunodiffusion method was used (Stojceski, Malpas & Witts, 1965).

It might be thought that the percentage saturation of the TIBC would show a similar close correlation with the iron stores. This is not so in normal subjects or in those with depleted stores but no anæmia (Weinfeld, 1965). In some subjects with early iron deficiency and a raised TIBC the serum iron level may actually rise and so maintain a normal percentage saturation. Further depletion of the stores is associated with a decreased percentage saturation due both to a reduction in serum iron level and a greater rise in TIBC.

### MAINTENANCE OF BODY IRON

This is dependant on a balance between loss of iron from the body, total intake and percentage absorption.

#### Loss

Iron is normally lost in the stools, urine and from the skin. Dubach, Moore & Callender (1955) measured faecal radioactivity in normal subjects at intervals up to 140 days after giving a tracer dose of radioiron parenterally. The calculated average daily faecal excretion was 0.3–0.5 mg. Some of the iron excreted is in desquamated mucosal cells and some is lost as hæmoglobin (Ebaugh *et al.*, 1958).

Iron is lost from the skin mainly in the form of desquamated cells although some iron is present in cell-poor sweat. Widely differing estimates of the total amount lost have been given. Dubach, Moore & Callender (1955) used plastic suits to collect the sweat during periods of  $\frac{1}{2}$ –2½ hours in a warm room. Up to a litre of sweat was obtained but even at this accelerated rate of sweating the calculated total loss was no more than 0.5–1 mg daily. Some iron is also lost in the urine but under normal conditions this is probably less than 0.1 mg daily.

Women also lose iron as a result of menstruation and pregnancy. The average menstrual loss of iron in normal subjects is 12 mg per period but there is a wide range (2–38). In any one individual, however, the amount lost in

successive periods is remarkably constant (Hyttén, Cheyne & Kloppe, 1964). The cost of a normal pregnancy in terms of iron loss to the mother also varies considerably. (Table 6.III.)

TABLE 6.III

THE IRON COST OF A NORMAL PREGNANCY (FROM MOORE, 1964)

<i>Iron Lost</i>	to foetus	201–372 mg
	in placenta and cord	34–170 mg
	in blood loss at delivery	100–250 mg
	in lactation for six months	100–180 mg
<i>Iron Saved</i>	by 15 months amenorrhœa	480–240 mg
NET COST		0–700 mg

Measurements of the total body loss of iron have been obtained by Finch (1959) who injected a tracer dose of the long-lived radioisotope,  $^{55}\text{Fe}$ , and followed the radioactivity in circulating hæmoglobin for periods of 46–54 months. After the first year the rate of decline was relatively constant and the total daily body loss averaged 0.61 mg in males and 1.22 mg in menstruating women. More recently Saito and co-workers (1964) used  $^{59}\text{Fe}$  and a whole body counter and found an average loss of 0.89 mg daily in male subjects.

In certain conditions not commonly associated with iron loss the amount excreted may be appreciably greater. In patients with proteinuria for instance the urinary iron excretion may be increased because of iron lost with transferrin (Rifkind *et al.*, 1961). In iron storage diseases there is an increased excretion of iron from gut, skin, urinary and respiratory tract as a result of desquamation of cells containing increased quantities of iron. This may be an important compensatory mechanism acting to maintain iron balance. One patient with transfusional siderosis studied by Crosby, Conrad & Wheby (1963) after his anæmia had been cured was calculated to have lost 3.5 mg daily over a period of 12 years. High dermal losses due to sweating have been thought to be an important factor in the high incidence of iron deficiency in tropical areas. Although such losses may be important initially there is good evidence that the amount lost in the skin in established iron deficiency is markedly decreased. (Hussain & Patwardhan, 1959.)

### Intake

Estimates for urban populations in the U.S.A. indicate that the average daily intake of iron in food has risen from 11.8 mg in 1936 to 17 mg in 1955 (Moore, 1964). In Britain the average diet provides about 14 mg daily (National Food Survey Committee, 1963). Most of these values have been derived from surveys of dietary habits rather than from chemical analyses of food being eaten. Consequently they do not include iron added to the food during its preparation. The very high intake of the Bantu is largely due to the use of iron cooking utensils.

The total daily intake will also depend on the amount of alcohol consumed. The iron content of American or French wines popular in the U.S.A. varies between 2.3 and 6.2 mg/l (MacDonald, 1964). The iron is derived mainly from the metal equipment used in preparation and storage and the

concentration was formerly very much higher. Beer and spirits contain little iron. Water particularly in the cities also has a low iron content, although concentrations of up to 5 mg/l have been found in water from some deep wells. Iron-containing tonics are another source of an increased intake that may be overlooked.

### Absorption

To maintain iron balance 1–1.5 mg need to be absorbed daily in children and male adults and 2–2.5 mg daily in menstruating women. The regulation of absorption at these levels despite variation in dietary intake and the rapid compensatory increase in absorption which occurs when additional iron is needed is dependent on the interaction of factors present in the lumen of the gut and in the mucosa (Fig. 62).

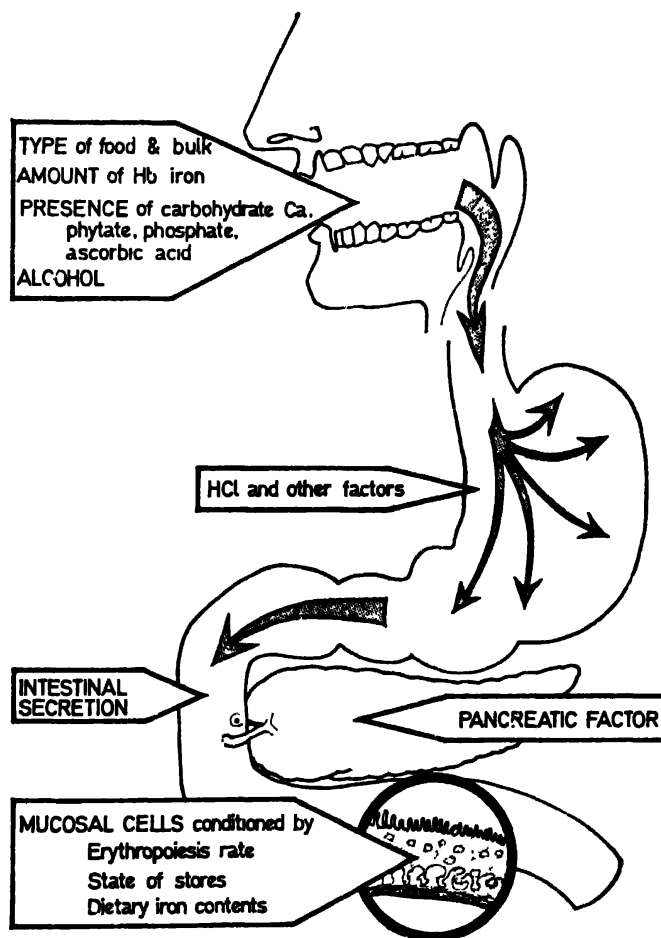


FIG. 6.2. Schematic illustration of factors involved in iron absorption.

### Intraluminal Factors

Iron is present in food mainly in the ferric state and in the form of various complexes. A variable amount is present as hæmoglobin. Classically iron is said to be released and reduced to the ferrous state before absorption in the free ionized form by the duodenal mucosa.

Studies of the absorption of biosynthetically labelled foodstuffs show that



the availability of iron in different foodstuffs varies widely. The percentage absorption is higher from hæmoglobin or muscle than from eggs and vegetables (Moore, 1964). Eggs may be a relatively poor source because the ferric iron they contain is strongly complexed to the phosphate of yolk phosphoproteins. The problem is a complicated one for the foods may interact. At the pH of the stomach ferric iron is reduced by certain protein foods and the presence of beef may increase the percentage absorbed from other constituents of the meal (Johnston, Frenchman & Boroughs, 1948). The presence of phosphates, phytates and calcium salts reduce the percentage absorption of iron salts and to a lesser extent the absorption of food iron (Bothwell & Finch, 1962). Ascorbic acid has the reverse effect.

Numerous studies have shown that the absorption of a labelled inorganic ferrous salt is considerably reduced if fed together with food. In one study in children the mean absorption of the ferrous salt alone was 27 per cent as compared with 17 per cent when it was given with 200 ml of milk and 5 per cent when given with food (Schulz & Smith, 1958). Whether this effect is related to the bulk of the meal or to the presence in the meal of blocking substances such as phytates is uncertain. Over one third of the daily intake of iron in this country is provided by bread and flour. Since 1953 white flour has been fortified with iron in the form of ferrum redactum, a variety of finely divided metallic iron to a minimum concentration of 1.65 mg/100 g flour. However, Elwood (1963) found that hæmoglobin levels did not rise with this bread even when the iron concentration was increased to 60 mg/100 g flour, although there was a rapid response to ferrous gluconate. It is possible though that the lack of effect was due to the form in which iron was given rather than to an effect of the bread.

Iron may be absorbed other than in the ferrous ionized state. The iron of hæmoglobin is probably absorbed intact in the hæm porphyrin ring. Its absorption unlike that of ferrous iron is unaffected by the addition of food, phytate or ascorbic acid (Turnbull, Cleton & Finch, 1962). It has also been shown that certain reducing substances such as fructose can form highly stable low molecular weight chelates with iron under certain conditions of concentration and alkaline pH (Charley *et al.*, 1963). Experimentally these sugar chelates facilitate the diffusion of iron across the membrane of the intestinal mucosal cells (Stitt *et al.*, 1962). The formation of chelates with carbohydrates as food iron enters the small intestine may explain earlier observations in animals that a high dietary intake of carbohydrate increases the accumulation of iron.

It would appear, however, from measurements of absorption in iron deficiency that when there is a need a large proportion of the dietary iron can be made available for absorption (Table 6.IV).

*Gastric and Pancreatic Secretion.* Although the importance of gastric acidity has been disputed two recent studies have shown marked differences in the absorption of food iron in subjects with achylia gastrica. Cook, Brown & Valberg (1964) found the mean absorption of a labelled ferrous salt given with bread was 19.8 per cent in treated patients with pernicious anæmia as compared with 32.5 per cent for the controls. The addition of normal gastric juice to the meal significantly increased the absorption in the achylia gastrica group; an effect not seen when the juice was previously neutralized. Goldberg,

**TABLE 6.IV**  
**ABSORPTION OF IRON IN CONTROL SUBJECTS AND IN PATIENTS WITH IRON DEFICIENCY ANÆMIA**

<i>Author</i>	<i>Form of Labelled Iron</i>	<i>Method of Measurement</i>	<i>Control Subjects % Mean Range</i>	<i>Iron Deficiency Anæmia % Mean Range</i>
Goldberg, Lochhead & Dagg (1963)	Ferric Chloride with standard meal	Single isotope modification	—	58 (17-89)
Callender (1964)	Ferrous Sulphate Hæmoglobin	Stool counting	30 (8-70)	56 (14-94)
		Stool counting	13 (1-32)	25 (1-68)
Moore (1964)	Biosyn- thetically labelled foods	Stool counting or utilization for Hb. synthesis	7 (1-36)	20 (1-62)
Pitcher <i>et al.</i> (1965)	Ferric chloride with standard meal	Double isotope technique	5 (1-13)	—
Turnbull (1965)	Ferric chloride with standard meal	Single isotope modification	3 (1-10)	54 (42-80)

Lochhead & Dagg (1963) found that the mean absorption of ferric chloride given with a standard meal in patients with iron deficiency anæmia was 57.5 per cent as compared with 18.5 per cent in controls with comparable iron deficiency but normal gastric acidity. Also Jacobs, Bothwell & Charlton (1964) showed that the addition of hydrochloric acid markedly increased the absorption of labelled ferric salts. They suggested that it acted by maintaining ferric iron in solution until it reached the duodenal mucosa. It is known that ferrous ions remain in solution at a much higher pH than do ferric ions.

Experimentally, pancreatic damage, induced either by ligating the duct or by ethionine, leads to an increased iron absorption and deposition in the liver. An increased absorption is also found in some patients with severe chronic pancreatitis (Davis & Badenoch, 1962) and may be decreased by pancreatin therapy. Pancreatic secretion appears, therefore, to contain a substance which inhibits absorption.

There may be other factors present in gastric and intestinal juice which regulate iron absorption. Murray (1965) measured the uptake of ferrous iron by everted sacs of rat mucosa which had previously been exposed to *neutralized* gastric or intestinal juice. When juice from subjects with iron deficiency anæmia or hæmochromatosis was used the uptake was considerably greater than when secretion from normal subjects was tested. The effect was abolished by heating to 65°F for 30 minutes beforehand. The relationship between this factor and hydrochloric acid which also has a stimulatory effect on absorption

and of pancreatic juice which inhibits absorption and which represents the main component of intestinal secretions at this level requires further study.

### **Role of the Mucosa**

The mucosal block theory of iron absorption proposed in the early 1940s later fell into disrepute. A number of workers showed that as the dose of iron salts or food iron was increased the net amount retained was greater even though the percentage absorption progressively decreased (Smith & Pan-naciulli, 1958).

Recent experimental studies in the rat have reaffirmed the role of the mucosa in regulating iron absorption. Crosby and co-workers (Conrad & Crosby, 1963; Wheby & Crosby, 1963; Conrad, Weintraub & Crosby, 1964) have shown that only a proportion of iron taken up initially by the mucosa reaches the plasma. The remainder is held within the mucosal cells and later excreted when the cells are shed into the lumen at the end of their life span. In iron overloaded animals the percentage of iron taken up by the mucosa is less and an increased amount is retained in the cell. In contrast in iron deficient animals the percentage of the dose absorbed is greater and most of it passes rapidly through the mucosa into the plasma. These workers also showed that if  $^{59}\text{Fe}$  was given parenterally radioactivity could be found in newly forming villus cells in the crypts of Lieberkühn. Less activity was found there in iron deficient animals. They suggested that the amount of "messenger" iron incorporated in these cells was dependent on the body's need for iron and determined the amount which they were subsequently able to absorb from the lumen. The increased incorporation of iron into the mucosal cells when the stores were overloaded also provided a means for increasing iron excretion.

Charlton and co-workers (1965) used immunological techniques to demonstrate that labelled ferritin was present in the mucosa after small doses of radioiron. They also found that the absorption of iron occurred in two phases. In the first iron was moved very rapidly from the lumen into the mucosa and could be isolated there in a non-ferritin bound fraction. Although not able to identify the nature of this fraction they showed that it did not consist of iron complexed to glycine and serine as suggested by Brown & Rother (1963). Some of this fraction was subsequently transported to the plasma. The rest was diverted to ferritin. There was some evidence in normal animals that the iron in this fraction could be subsequently released to the plasma. In iron overloaded animals the amount taken up by the mucosa was decreased and there was also increased formation of ferritin. When large doses of iron were given orally the proportion of activity taken up by the mucosa and diverted to the ferritin fraction was reduced suggesting that the regulating mechanism was only efficient when the intake was within the normal range.

Manis & Schachter (1964) used everted duodenal sacs *in vitro* and isolated duodenal loops *in vivo* to measure the divalent and trivalent iron pools forming in the mucosa during these two phases of absorption. They showed that iron was rapidly absorbed from the lumen into a divalent pool most of which was subsequently transported to the blood. During this time a trivalent pool formed slowly in the mucosa and subsequently when uptake of iron from the lumen had ceased there was slow increase of the trivalent iron

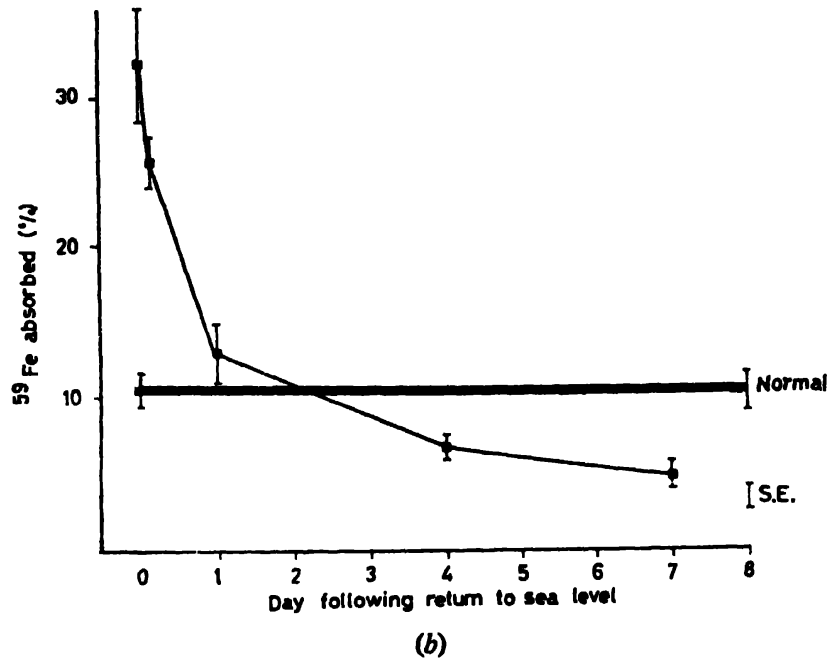
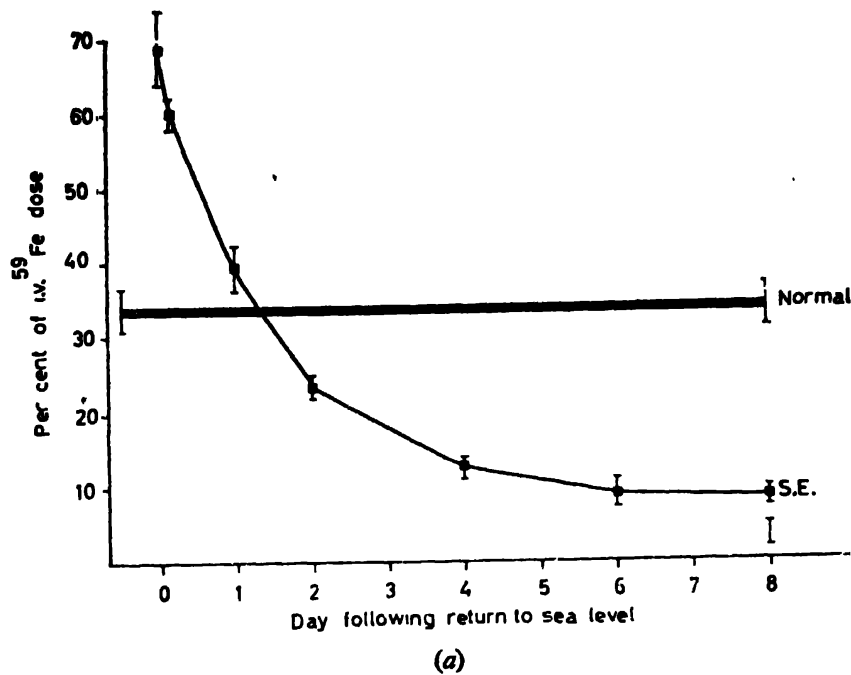


FIG. 6.3. Red cell incorporation of  $^{59}\text{Fe}$ , (a) and absorption of  $^{59}\text{Fe}$  ferrous sulphate, (b) in rats on return to sea level after 7 days at 20,000 ft (from Weintraub, Conrad & Crosby, 1965).

at the expense of that present in the divalent form. This trivalent pool may be available for subsequent transport into the blood but probably only after reconversion to the divalent form. When iron overloaded animals were examined the initial velocities both of uptake and transport of divalent iron were significantly decreased and were associated with an increased transfer to the trivalent pool. Ferritin, however, appeared to play little part in these

regulatory processes for estimations of apoferritin content showed that this would not account for more than 8 per cent of the trivalent iron.

*Influence of Iron Stores and Rate of Erythropoiesis.* Numerous studies on man and animals suggest that the amount of iron absorbed is dependent on the state of the stores and the rate of erythropoiesis. The latter appears to be the more important factor or at least has an overriding influence. Increased absorption can be shown in iron overloaded animals following acute blood loss despite the presence of the increased stores (Weintraub, Conrad & Crosby, 1964a).

Erythropoiesis is believed to affect absorption by producing changes in plasma iron turnover which in turn determine the amount of "messenger" iron incorporated in the developing intestinal mucosal cells. In man there is a four-five day lag period following acute hæmorrhage before absorption increases and this can be correlated with the onset of an increased plasma iron turnover (Weintraub, Conrad & Crosby, 1964b). The close relationship between erythropoiesis, plasma iron turnover and iron absorption was also shown by Weintraub, Conrad & Crosby (1965) in animals exposed to a low atmospheric pressure. This induced a physiological polycythæmia with an increased rate of erythropoiesis. Absorption also increased rapidly and showed an equally rapid fall on return to normal atmospheric pressure, these changes closely following alterations in red cell production rate (Fig. 6.3). The decreased absorption on return to atmospheric pressure was associated with an increased concentration of iron in the mucosa estimated chemically, and with an increased incorporation of intravenously administered radioiron.

*Effect of an Iron-deficient Diet.* Recent studies in the rat have confirmed early observations that absorption increases after four-five days on a low iron diet (Pollack, Kauffman & Crosby, 1964; Charlton *et al.*, 1965). This is not due to the small net loss of iron whilst on the deficient diet and the plasma iron turnover is not significantly altered. The gradual increase in absorption found is compatible with the steady replacement of absorbing cells in the intestinal villi by new cells conditioned in some way during their formation in the crypts. However, Rush, Figallo & Brown (1965) could find no increase in absorption in man after a week on a low iron diet and further studies are needed.

Finally, it is to be stressed that the ability of the mucosa to regulate absorption in accordance with the body's needs is probably only operative within certain narrow limits of dietary iron content. When the intake is abnormally high, although the percentage absorption decreases, the net amount absorbed is increased even though the stores may already be considerably overloaded.

### MEASUREMENT OF ABSORPTION IN MAN

The advent of radioiron led to a rapid abandonment of the earlier chemical balance studies used for measuring iron absorption. Nevertheless, although these balance techniques are tedious both for investigator and patient they remain the only means of truly assessing the iron retention from any mixed diet. In four carefully controlled balance studies the mean absorption from a basic diet varied from 11-14 per cent (see Mason, 1964). This figure

is consistent with the maintenance of normal iron balance whereas many of the values reported with radioiron techniques are not (Table 6.IV).

The main problem lies in the administration of the tracer dose.  $^{59}\text{Fe}$  for instance can be used to label a solution of an inorganic ferrous or ferric salt which is then given to a fasting patient. It is clear from the earlier discussion that the values obtained will give little indication of the absorption of food iron. Another technique is to incorporate the label biosynthetically into individual food items such as eggs, meat and vegetables during their production. Problems arise in the quantitation of the radioiron dose and the method can only be used to measure absorption from one food item at a time. Labelled hæmoglobin has been used extensively, this being easy to prepare and standardize. Hæmoglobin, however, represents only one form of dietary iron and has a distinct mode of absorption. Another technique is to give the patient a solution of a labelled iron salt to drink during the course of a standard meal. The tracer dose is believed to mix with a large proportion of the dietary iron present. Results agree well with the range of values found for the absorption of biosynthetically labelled foods and are also in agreement with the findings of chemical balance studies (Table 6.IV). It is possible though that the proportion of the tracer dose complexed to food iron varies according to the bulk and composition of the standard meal chosen (Turnbull, 1965).

The percentage absorption of the radioiron dose, however administered, can be determined in four ways.

### **Stool Counting**

Absorption is derived indirectly from the difference between the dose given and the total amount excreted in the stools over a period of five–seven days. Consistently reliable stool collections are difficult even in hospital and the loss of one stool in subjects with a low absorption will make a large difference to the calculated result.

### **Whole Body Counting**

The patient is counted five hours after administration of the dose and at intervals up to ten days until body activity is at a plateau. Absorption is therefore determined directly and only small doses of radioactivity need be administered. Unfortunately whole body counters are expensive although cheaper models suitable for measuring iron absorption have been described (Pitcher *et al.*, 1965).

### **Double Isotope Technique**

The other isotope of iron,  $^{55}\text{Fe}$ , is used to label the oral dose and at the same time as this is given  $^{59}\text{Fe}$  is injected intravenously. Fourteen days later a blood sample is taken and the activity of the two isotopes in the red cells determined. The activity of  $^{59}\text{Fe}$  indicates the percentage of plasma iron used for hæmoglobin synthesis and it is assumed that the percentage utilization of the absorbed  $^{55}\text{Fe}$  will be the same. Thus if 50 per cent of the  $^{59}\text{Fe}$  appears in the red cells then the activity of the  $^{55}\text{Fe}$  there represents 50 per cent of that actually absorbed. The counting of the two isotopes in the blood samples is technically complex for the iron must be separated from the blood by digestion and precipitation followed by electroplating onto metal planchettes. Pitcher

and co-workers (1965) have recently described modifications to the original method which make it simpler and more reliable and also enable it to be used for both single and serial measurements of iron absorption. They found good agreement between the double isotope technique and the whole body counter (Fig. 6.4) but less agreement with stool counting.

The method is based on the assumption that transferrin bound iron injected intravenously is treated identically as far as hæmoglobin synthesis is concerned to iron reaching the circulation via the gut and portal vein. It has been suggested that in patients with increased saturation of the serum iron-binding capacity some isotope is lost in its first passage through the liver. This indeed occurs when the transferrin saturation is raised experimentally by intravenous infusions of iron to over 70 per cent in dogs and to 100 per cent

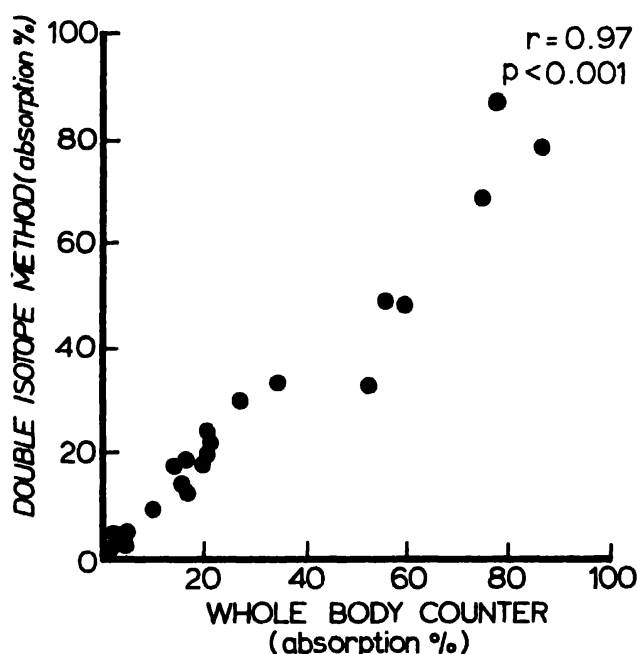


FIG. 6.4. The relation between results obtained with the double isotope technique and those found by whole body counting done simultaneously (from Pitcher *et al.*, 1965).

in man (Wheby & Umpierre, 1964). However, Pitcher and co-workers (1965) found excellent agreement between the double isotope technique and the whole body counter in patients with a percentage saturation greater than 70 per cent and in whom considerable iron overload was present. Thus although there may be a transient hold up in the liver in such cases, there is little loss of isotope in terms of red cell utilization at 14 days.

#### Single Isotope Modification

To obviate the need for digestion and electroplating the double isotope technique has been split into two parts so that only the easily counted  $^{59}\text{Fe}$  is used (Goldberg, Lochhead & Dagg, 1963; Turnbull, 1965).  $^{59}\text{Fe}$  is first given by mouth and 14 days later the percentage appearing in the red cells determined. An intravenous dose of  $^{59}\text{Fe}$  is then given and its incorporation into the red cells measured after another 14 days. The absorption of the first

dose can then be calculated in the same way as when two isotopes are given simultaneously. Unfortunately, a single measurement of absorption takes 28 days and the method is dependent on the patient remaining in a steady state.

### IRON DEFICIENCY

The presence of hypochromia and microcytosis of the circulating red cells is often considered essential for the diagnosis of iron deficiency anaemia. An inadequate iron supply to the marrow may, however, retard erythropoiesis for weeks or months before these characteristic abnormalities occur and the anaemia when it first appears is usually normochromic and normocytic.

Recent studies have clarified the sequence of events leading from the initial early depletion of iron stores to the final classical picture of a hypochromic anaemia. Weinfeld (1964) found an early rise in the serum total iron-binding capacity. This occurred when the iron content of liver and marrow as measured chemically was reduced to approximately a third, although marrow iron assessed histochemically was usually absent. With further depletion of the stores the sideroblast count began to fall. In normal subjects about 54 per cent of the normoblasts contain visible haemosiderin granules, whereas in iron deficiency the count falls to 0–12 per cent. Subsequently the serum iron level falls to below 60  $\mu\text{g}/100\text{ ml}$  and the transferrin saturation becomes markedly reduced. The supply of iron to the marrow then becomes insufficient and the formation of haemoglobin is retarded with ensuing anaemia. According to Bainton & Finch (1964) the transferrin saturation decreases at an early stage and when it falls to below 16 per cent there is insufficient iron available for proper marrow function and the formation of sideroblasts.

The final picture depends not only on the amount of iron supplied to the marrow but on the extent of marrow hyperplasia. This is less marked in iron deficiency anaemia than in other disorders of haemoglobin synthesis. The number of erythroid cells in the marrow as judged from the erythroid-myeloid ratio is half to twice normal as compared with values of 8–10 for thalassaemia. The degree of hypochromia and microcytosis in the peripheral blood film may be minimal in a patient with iron deficiency and a relatively hypocellular marrow, whereas patient with polycythaemia rubra vera who are iron deficient may show marked changes (Bainton & Finch, 1964).

### Iron Deficient Erythropoiesis

This term is of value in drawing attention to certain conditions in which haemopoiesis may be impaired as a result of an inadequate iron supply to the marrow even though the total body iron may be normal or increased. In 34 of the 122 patients with anaemia due to chronic infection or malignancy studied by Bainton & Finch (1964) the peripheral film showed significant hypochromia and microcytosis. The serum iron level, transferrin saturation and sideroblast count were reduced to a similar extent as in iron deficiency anaemia due to blood loss. Yet examination of the marrow showed normal amounts of storage iron and there is presumably a block in the release of iron from the reticulo-endothelial system.

Iron deficient erythropoiesis may be present in polycythaemia rubra vera



even though the total body iron content is increased. Inadequate availability of iron is sometimes a factor limiting the hæmopoietic response to anoxia in emphysema. Fielding & Zorab (1964) showed that the circulating hæmoglobin mass rose in 6 of 11 patients with severe bronchitis and emphysema treated with intramuscular iron-sorbitol.

### **Iron Deficiency without Anæmia**

Experimentally iron deficiency produces a deficiency of the cellular enzymes catalase, cytochrome and succinic dehydrogenase. Consequently it has been suggested that impaired cellular function is the cause of certain clinical syndromes seen in patients with iron deficiency but no anæmia. A double blind controlled trial of iron therapy in women with symptoms of fatigue, anorexia, palpitation and vertigo, in whom marrow hæmosiderin was absent although the hæmoglobin level was normal, showed significant improvement (Beutler, Fairbanks & Fahey, 1963).

Stafford (1961) found evidence of iron deficiency as shown by a low serum iron level in cases of excessive post-extraction dental bleeding, angular stomatitis, bruising and buccal ulceration. Iron deficiency without anæmia may also cause or at least aggravate functional menorrhagia. Seventy-four of 98 patients with a low serum iron level studied by Taymor, Sturgis & Yahia (1964) showed a significant decrease of the menorrhagia with iron therapy.

Iron deficiency in the female population is probably even more common than previously supposed. Fielding, O'Shaughnessy & Brunström (1965) used the differential ferrioxamine test to measure total chelatable body iron and found that 35 per cent of women with a normal hæmoglobin level had values in the same range as found in iron deficiency anæmia.

### **Œsophageal Web, Gastric Atrophy, Steatorrhœa**

Angular stomatitis and superficial glossitis are often associated with iron deficiency anæmia and respond to iron therapy. The association of anæmia with dysphagia and a postcricoid web, the Plummer-Vinson syndrome, is also well known. The dysphagia sometimes improves with iron therapy and it has been suggested that the œsophageal lesion is also the result of long continued iron deficiency.

Recent studies from Cardiff suggest a different pathogenesis. Elwood and co-workers (1964) determined the prevalence of postcricoid dysphagia in a population consisting of 2,188 men and 2,617 women, aged 40–75, living in three areas in the Rhondda Fach. Five per cent of the women gave definite evidence of dysphagia of at least two years' duration. Most of the women with dysphagia were examined by barium swallow and of these 15 per cent proved to have a postcricoid lesion. The figures in the males were lower. Hæmoglobin and serum iron levels were not significantly different in men or women with dysphagia, those with webs and a group of matched controls. There was therefore no evidence that iron deficiency was responsible for the œsophageal lesion. Two of the patients with webs had pernicious anæmia and the incidence of atrophic gastritis, as shown by a reduced serum pepsinogen level, was higher in this group.

The frequent association of œsophageal webs with an atrophic gastritis

was also shown by Jacobs & Kilpatrick (1964) who found histaminefast achlorhydria in 37 of 48 patients with a previously diagnosed Plummer-Vinson syndrome attending hospital. In 25 patients the absorption of Vit. B<sub>12</sub> was subnormal and two patients had overt pernicious anæmia.

Thus it would appear that iron deficiency is not the cause of the œsophageal lesion but is a secondary effect resulting from the atrophic gastritis and achlorhydria so frequently present. As already discussed lack of gastric acidity undoubtedly impairs the absorption of iron and patients with a chronic gastritis may also bleed intermittently which will further contribute to the development of an iron deficiency anæmia.

The ætiology of the œsophageal and gastric mucosal lesion is uncertain. Recent studies suggest that the gastric changes may be due to a primary defect in immune tolerance. The serum of patients with gastric atrophy and pernicious anæmia contains antibodies to intrinsic factor and to the parietal cells. In gastric atrophy without pernicious anæmia intrinsic factor antibody is absent and the incidence of the parietal cell antibody is lower although still significantly higher than in controls (Taylor, 1965).

A malabsorption state has been described in infants which is associated with iron deficiency and is reversible with iron therapy (Naiman *et al.*, 1964). Impaired absorption of iron is of course extremely common in idiopathic steatorrhœa and occasionally patients are described in whom this is present without an increased fæcal fat excretion (McGuigan & Volwiler, 1964).

#### **Anæmia after Partial Gastrectomy**

Frank anæmia develops in approximately a quarter of patients after partial gastrectomy and up to 50 per cent may show some hæmatological abnormality. The anæmia is usually of the iron deficiency type.

Although patients after a partial gastrectomy can absorb iron salts as well as normal subjects the absorption of hæmoglobin iron (Turnberg, 1966) and food iron is probably impaired. Turnbull (1965) found that patients with an iron deficiency anæmia after partial gastrectomy absorbed less radioiron given with a standard meal than patients with iron deficiency due to other causes, the mean values being 30.1 and 46.2 per cent respectively. The impairment in absorption was of the same degree as found in control subjects with iron deficiency anæmia and achlorhydria and the patients after partial gastrectomy in whom acid secretion was retained had higher absorptions than those with achlorhydria. Patients after partial gastrectomy who were not anæmic absorbed more radioiron than did healthy controls possibly because of suboptimal iron stores and it is clear that the impairment of absorption shown in the anæmic patients cannot be the primary cause of the iron deficiency. It seems unlikely that a reduction in dietary intake could account for a negative iron balance.

There is some evidence that patients lose blood intermittently after partial gastrectomy. Although occult blood tests in the fæces are positive in only a few instances, this does not exclude the possibility of intermittent losses of blood since it is known that such tests rapidly become negative after hæmorrhage has ceased. The most likely site of bleeding is the lower œsophagus. Williams (1965) finds that œsophagitis with a readily bleeding mucosa is quite commonly seen on endoscopy after partial gastrectomy whereas there

is little evidence of hæmorrhage from the stomach or jejunum. Windsor (1964) found radiological evidence of œsophageal reflux in 40 per cent and symptoms of œsophagitis in 26 per cent of post-gastrectomy patients most of whom were anæmic.

### Treatment

The idea that iron administered parenterally leads to a more rapid rise in hæmoglobin level than iron given orally seems to be increasingly prevalent. The recent findings of McCurdy (1965) are salutary. He compared iron-sorbitol and iron-dextran given intramuscularly with oral iron therapy in matched groups of patients with iron deficiency anæmia. The rate of rise during the first two weeks of therapy was identical (Fig. 6.5) and whereas each

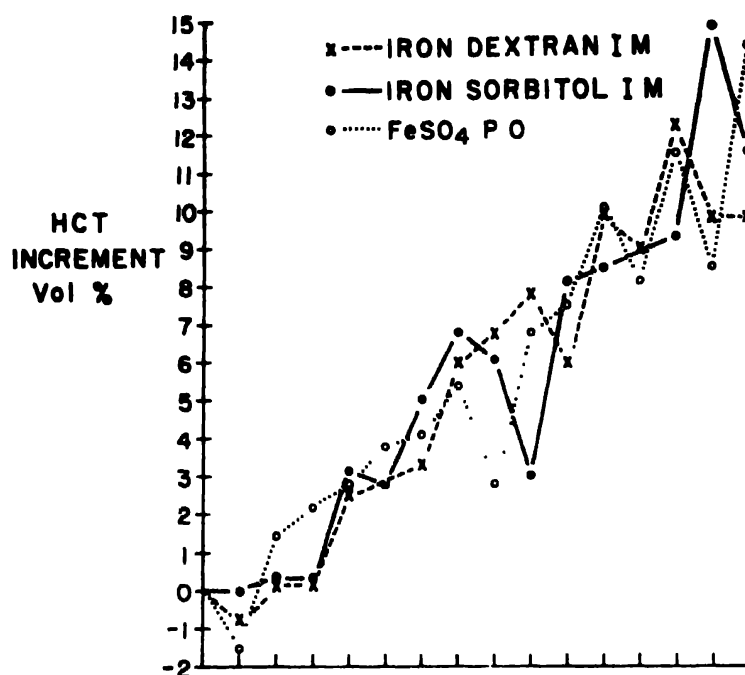


FIG. 6.5. Hæmatocrit increment versus time in days (indicated on bottom line of graph) in 3 comparable groups of patients with iron deficiency anæmia. FeSO<sub>4</sub> P O signifies ferrous sulphate given orally (from McCurdy, 1965).

of the parenteral preparations produced some side effects there were no adverse reactions to the oral iron.

There has been confusion in the past concerning oral iron therapy and occult blood tests. Illingworth (1965) studied the effect *in vitro* and *in vivo* of a variety of iron preparations on a series of tests done with "Occultest" and "Hæmatest" tablets and with the benzidine reaction. Ferrous sulphate, gluconate or succinate, iron and ammonium citrate all gave negative results whilst the more recently introduced and considerably more expensive ferrous fumarate and carbonate produced a high incidence of false positive tests.

### HÆMOCHROMATOSIS

Different workers have, unfortunately, used different criteria in the diagnosis of hæmochromatosis and confusion has also arisen because many cases

of cirrhosis of alcoholic or other ætiology show some increase in hepatic iron deposition. Also there is an increased incidence of the various clinical signs of hæmochromatosis, such as pigmentation, diabetes and gonadal atrophy, in cirrhosis whether or not there is excess iron. In the author's view the term hæmochromatosis should be restricted to cases of cirrhosis in which there is massive iron accumulation in the liver. Such cases will also show deposits of iron in other parenchymal organs such as pancreas, thyroid, adrenals and heart. The majority will be pigmented and have hepatomegaly but diabetes and gonadal atrophy are unlikely to be present in more than 60 per cent.

Although there is currently considerable controversy as to the ætiology of "idiopathic" hæmochromatosis it is convenient to separate these cases from those in which the development of hæmochromatosis appears to be a secondary phenomenon (Table 6.V).

TABLE 6.V

## CLASSIFICATION OF HÆMOCHROMATOSIS

1. Primary idiopathic hæmochromatosis
2. Secondary hæmochromatosis
  - rarely in* (a) cirrhosis with siderosis
  - (b) Bantu siderosis
  - (c) chronic refractory anæmia
  - (d) congenital transferrin deficiency

**Primary "Idiopathic" Hæmochromatosis**

The long held view that the disorder was due to an inborn error of metabolism causing increased absorption and slow accumulation of iron with eventual tissue damage was not seriously questioned until 1960 when MacDonald & Mallory proposed that it was simply a variant of a nutritional or alcoholic cirrhosis. They noted the finding of hæmosiderin deposits in 50–80 per cent of cases of portal cirrhosis, the frequent association of alcoholism with hæmochromatosis and the experimental evidence that iron overloading alone does not produce a typical pigmentary cirrhosis. Subsequently MacDonald and his colleagues (MacDonald, 1964) showed that certain alcoholic drinks, particularly wine, had a high iron content and they proposed that an increased iron intake in this form or resulting from the use of iron cooking utensils, or from the prolonged taking of iron containing tonics, could easily account for the amount of iron found deposited in the tissues. The cirrhosis in their view is unrelated to the iron deposition and results from the independent action of factors such as malnutrition, alcoholism or viral hepatitis. Recently they have shown that the prolonged feeding of rats with a choline deficient diet and added iron produces the typical picture of a hæmochromatotic cirrhosis together with extrahepatic parenchymal iron deposition, the latter apparently being due to an induced folic acid deficiency (MacDonald & Pechet, 1965b; MacDonald, Jones & Pechet, 1965).

Although MacDonald's work has been of inestimable value in drawing attention to the importance of environmental factors in the development

of excess iron deposition there is also much evidence in favour of a genetic defect.

### **Familial Involvement**

At the time of Peterson's review (1960) there were 25 well documented instances of the fully developed disease occurring in two or more siblings and five where it affected successive generations. Reports of particular interest since then include that of Dillingham (1960) who described four cases in one family and that of Johnson, Bridgeport & Frey (1962) who studied a large family which included two siblings with proven hæmochromatosis and in which there was evidence of transmission of severe hæmosiderosis through three generations. More recently Lloyd, Powell & Thomas (1964) have reported a family with hæmochromatosis in four members of two generations.

It is certainly true that the incidence of familial involvement is low in relation to the total number of cases reported. Finch & Finch (1955) could find only seven histologically proven examples of familial involvement in a series of 707 cases collected from the literature. Three factors may account for this low incidence. Firstly, hæmochromatosis does not usually present until middle life and even then may only be diagnosed during an unrelated infection or from a routine examination. The simple taking of a family history is therefore not sufficient to assess familial involvement. Secondly, a number of families have been reported in which young children have been found to have severe hæmosiderosis (Perkins *et al.*, 1965). It seems likely that some of these children will eventually develop hæmochromatosis, although at present these families cannot be regarded as examples of familial involvement. Thirdly, there is the tendency of some workers to consider all cases of portal cirrhosis with iron deposition even if slight and apparently secondary as examples of hæmochromatosis (MacDonald, 1964). Their inclusion will lead to a falsely low overall incidence.

*Systematic Family Studies.* These have revealed a high incidence of isolated signs of the disease such as pigmentation, hepatomegaly or a raised serum iron level (Debré *et al.*, 1958; Morgan, 1961).

Liver biopsy is the best method for assessing involvement of relatives for it shows whether tissue damage is present in addition to excess iron. The detailed studies of Pirart & Gatez (1958), Bothwell and co-workers (1959), Brick (1961), Johnson, Bridgeport & Fry (1962), Williams, Scheuer & Sherlock (1962), Ploem and co-workers (1965) indicate that a considerable proportion of close relatives have significant hepatic siderosis, this being found both in the same and in successive generations. Williams, Scheuer & Sherlock (1962) found excess free iron in liver biopsy sections from 28 of 46 relatives examined although clinical signs of the disease were slight and only one relative had a definite cirrhosis. They suggested that the disorder might be inherited as an intermediate characteristic and that the mildly affected relatives represented the heterozygous state. Such a mode of inheritance would be consistent with the much more frequently described clinical involvement of siblings than of children and is also in accord with the occasional reports of consanguinity in the parents.

MacDonald (1964) has suggested that the occurrence of hæmosiderosis in the relatives is simply due to common environmental factors and is a

reflection of the high incidence of hæmosiderosis which he has found in various autopsy and biopsy series. However, other series which as described earlier are more representative of the general population indicate that only about a quarter of normal subjects have stainable iron in the liver.

Powell (1965) estimated the extent of the abnormal iron stores by measuring urinary iron excretion after injection of a chelating agent. Of the 63 relatives of patients with idiopathic hæmochromatosis examined 16 had an abnormal result, whereas only one out of 34 relatives of patients with an alcoholic cirrhosis and a secondary siderosis had increased stores. The affected relatives fell naturally into two groups; one with grossly excessive iron stores and hepatic fibrosis or cirrhosis (12.5 per cent of siblings), the other with hæmosiderosis and a slight to moderate increase in total body stores (28 per cent of siblings). This finding also supports an intermediate form of inheritance.

Evidence against such an inheritance is the description of an unusually severe juvenile form of the disease which has been thought to represent the homozygous state (Debré *et al.*, 1958). However, in none of the reported cases have the parents shown clinical signs of hæmochromatosis. Also Perkins and co-workers (1965) in a review of 10 reports of idiopathic hæmochromatosis in subjects under 20 years of age found that the frequency of abnormal iron storage in the families was similar to that described in the families of patients presenting at a later age.

The mode of inheritance is not merely one of academic interest for if the disorder is inherited as an intermediate characteristic then only one out of every three "affected" relatives is liable to develop the full disease, and it is these relatives that need to be diagnosed and treated at an early stage.

### Iron Absorption

A major criticism of the genetic ætiology has been the failure of a number of investigators to find increased absorption. Raised values are reported in only 3 of 15 cases in the literature where absorption had been measured using a ferrous salt and stool counting (Williams & Pitcher, 1963).

Williams and co-workers (1966) have recently reported the results of serial measurements in 19 patients with idiopathic hæmochromatosis (Fig. 6.6). Absorption was normal or only marginally increased at the time of presentation but increased progressively during venesection therapy. In five of eight patients examined at intervals up to six years after completion of treatment the absorption subsequently decreased although in only one did it return to normal. In three it was maintained at a high level. These findings are consistent with a primary defect in absorption which had been depressed at the time of presentation as a result of saturation of the stores. Alternatively, the increased absorption might be secondary to the presence of cirrhosis, existence of portal-systemic collaterals or coincident pancreatic damage all of which may, as is described later, affect iron absorption. In the patients studied, however, the cirrhosis was inactive by all criteria and none had a large collateral circulation. There was also no correlation between absorption and the presence of pancreatic damage as evidence by diabetes.

Other evidence in favour of a genetic defect in the regulation of iron absorption is the finding by Williams and co-workers (1965) of an unexplained

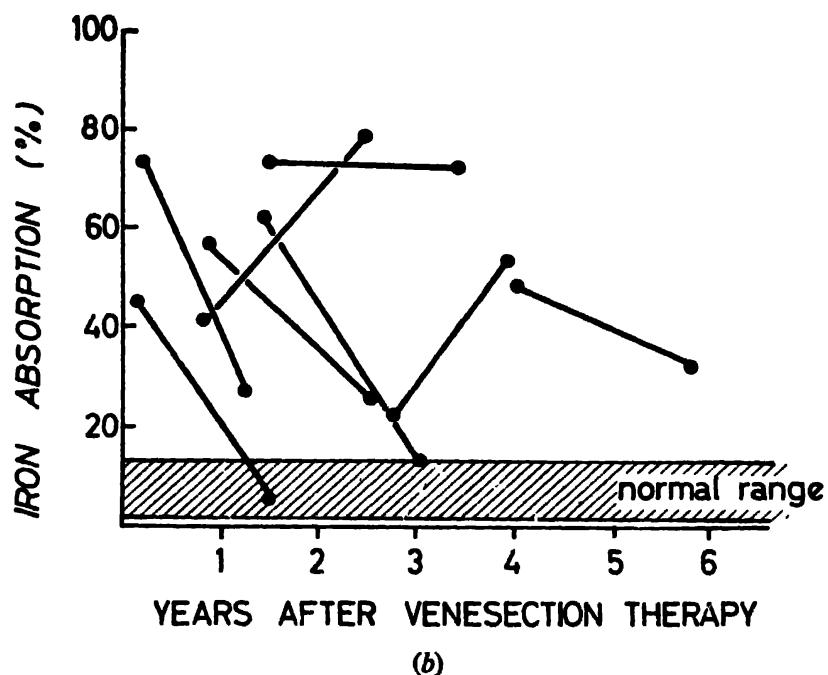
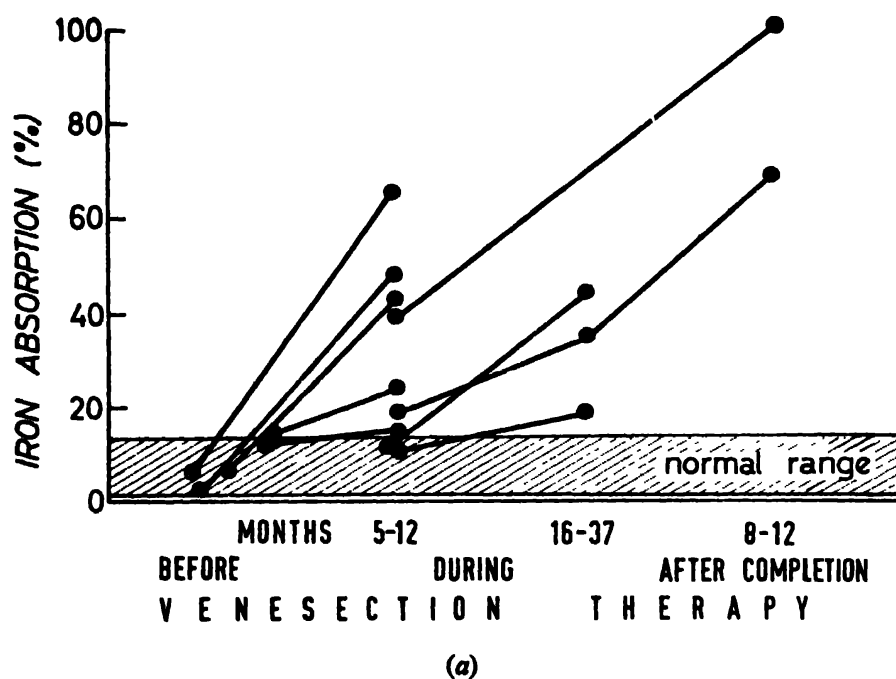


FIG. 6.6. Serial readings of iron absorption in 9 patients with idiopathic haemochromatosis examined at various intervals in relation to venesection therapy (a) and in 8 other patients examined after completion of treatment (b) (from Williams *et al.*, 1966).

increase in absorption in 59 per cent of 29 close relatives of patients with idiopathic haemochromatosis (Fig. 6.7).

Only a small increase in absorption, if maintained, is necessary to produce the amount of excess iron found at the time of presentation in middle life. What is needed is knowledge of the balance between absorption and loss in

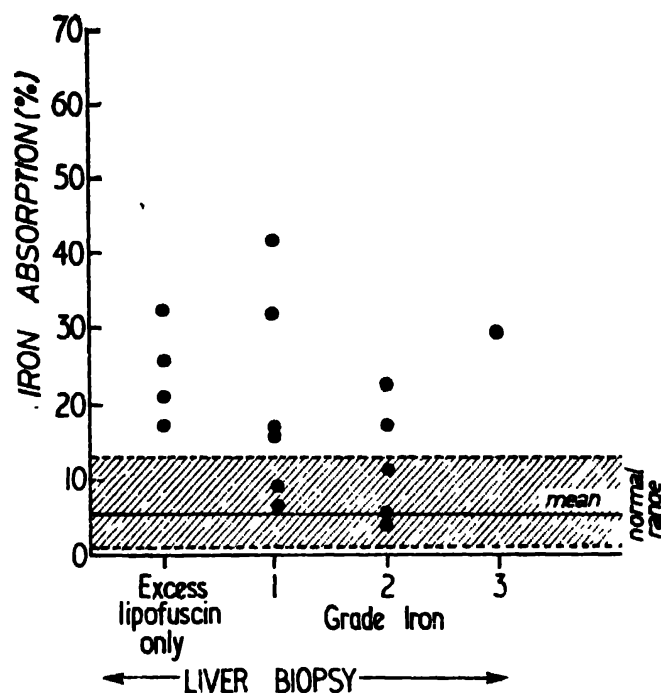


FIG. 6.7. Iron absorption measurements in 16 relatives of patients with idiopathic hæmochromatosis related to the abnormalities in liver biopsy sections (from Williams *et al.*, 1965).

the individual patient rather than a comparison of his absorption value with the inevitably wide range found in control subjects.

### Miscellaneous

In most series of patients with idiopathic hæmochromatosis there are a considerable number of cases who do not appear to have drunk excessively and in whom no other sources of an excess oral intake can be found. This incidence of alcoholism was 29 per cent in the series of Finch (1955) from America, 31 per cent in that of Williams, Scheuer & Sherlock (1962) from England and 44 per cent in Powell's (1965) series from Australia.

The overlap histologically between idiopathic hæmochromatosis and alcoholic cirrhosis with a secondary siderosis is also not as great as has been suggested. In only eight of the 116 cases of cirrhosis examined by Zimmerman and co-workers (1961) were the hæmosiderin deposits heavy enough to cause difficulty in distinguishing the lesion from idiopathic hæmochromatosis. Similarly in a series of 161 consecutive liver biopsies (Scheuer, Williams & Muir, 1962) although some siderosis was found in over half the cases of alcoholic liver disease in only one did it approach that seen in hæmochromatosis.

The hepatic lesion of idiopathic hæmochromatosis also appears to differ in a number of respects from that of a primarily alcoholic cirrhosis. Hepato-cellular impairment and BSP retention are more marked in patients with alcoholic cirrhosis. Also portal hypertension is present more commonly in this group.

The response to venesection therapy also suggests a causal relationship of the iron deposition to tissue damage. Eleven of the 36 cases seen



personally by the author since 1959 and treated by venesections have died and in six of these death was due to an unrelated cause. Therapy has been accompanied by marked clinical improvement with disappearance of pigmentation, hepatomegaly and in some cases improvement in glucose tolerance. One patient with hæmochromatosis has been described in whom serial biopsies showed reversal of the cirrhosis (Knauer, Gamble & Monroe, 1965).

Thus there is much evidence implicating a genetic defect in primary hæmochromatosis. What is not clear at present is whether the transition from hæmosiderosis to hæmochromatosis is due to variation in genetic dose or whether environmental factors particularly alcoholism are involved. Careful follow up of "affected" relatives should help to solve this problem. Although some physicians recommend that all affected relatives should be treated by venesections in the author's view such therapy is only indicated if there is evidence of tissue damage as well as marked siderosis.

### Secondary Hæmochromatosis

#### Cirrhosis with Siderosis

In occasional cases the siderosis may approach that seen in idiopathic hæmochromatosis. Sabesin & Thomas (1964) have reported two well documented cases which at necropsy showed all the pathological features of primary hæmochromatosis but in whom an earlier biopsy had revealed portal cirrhosis only with minimal iron deposition.

A number of factors may be concerned in the development of siderosis in cirrhosis. Measurements done by a variety of methods have shown that a proportion of patients with cirrhosis have an increased iron absorption. This may be found even though iron stores are already increased (Conrad, Berman & Crosby, 1962; Greenberg *et al.*, 1964). In the series of patients with cirrhosis and a normal serum iron shown in Fig. 6.8 absorption was increased in about a third, the values being comparable to those obtained in other cirrhotic patients with definite bleeding or iron deficiency.

The increased absorption in cirrhosis may be due to associated pancreatic damage for in a number of cases absorption has decreased following administration of pancreatin or duodenal juice (Callender & Malpas, 1963; Linscheer *et al.*, 1964; Davis & Biggs, 1964). Histological evidence of chronic pancreatitis can be found at autopsy in a high percentage of patients with cirrhosis of alcoholic ætiology (Sobel & Waye, 1963) and in life Van Goidsenhoven and co-workers (1963) showed abnormalities in pancreatic function in over half their patients with cirrhosis. Pancreatin administration, however, has also been observed to decrease absorption in a non-cirrhotic patient with simple iron deficiency (Callender, 1964).

The increased absorption might also be related to the hæmolysis so commonly present in liver disease or to interference with normal iron utilization due to deficiency of folic acid, Vit. B<sub>12</sub> or pyridoxine (Pitcher & Williams, 1963; Sullivan & Herbert, 1964; Kimber *et al.*, 1965).

The presence of liver damage per se may increase absorption. In ethionine induced liver cirrhosis the damaged cells have a temporarily increased affinity for iron due to the presence of a PAS positive material (Kent, 1965). Rats fed a choline deficient diet with added iron absorb more iron than rats

fed a normal diet with the same amount of added iron (MacDonald & Pechet, 1965b).

In addition to providing a source for an increased intake alcohol may also have an effect in stimulating iron absorption. Charlton and co-workers (1964) have shown that whisky or brandy increases the absorption of ferric iron in fasting subjects probably as a result of stimulation of gastric acid secretion.

*The Effect of Portal-systemic Venous Shunts.* A number of patients with cirrhosis have been reported in whom hæmochromatosis has developed after shunt operations, a liver biopsy at the time of operation having shown no excess iron deposition (Tisdale, 1961; Schaefer *et al.*, 1962). Experimental

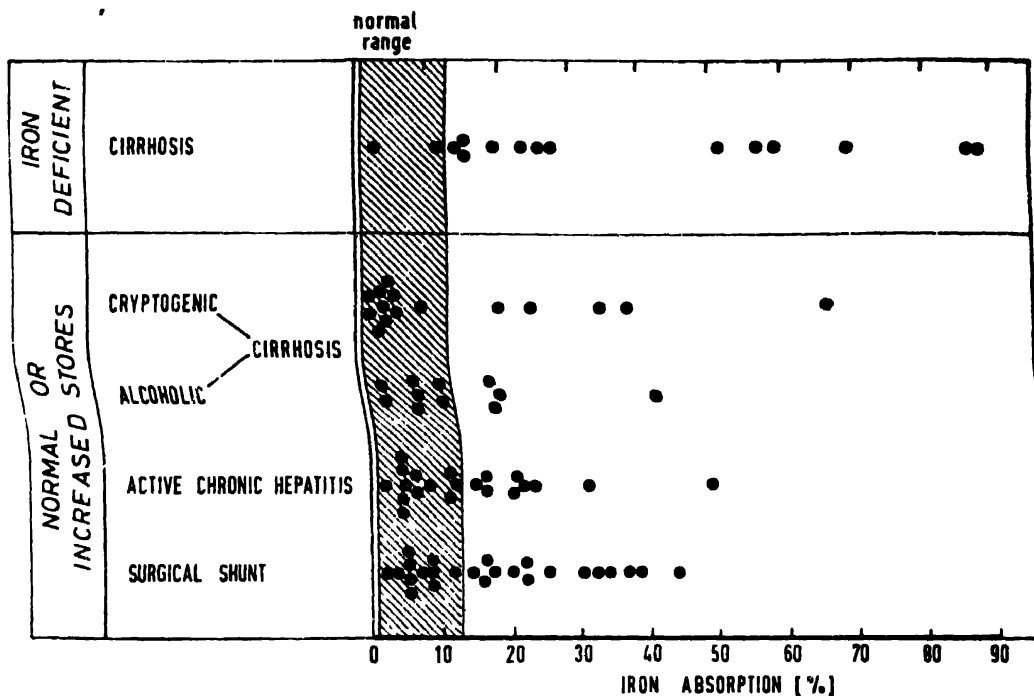


FIG. 6.8. Iron absorption values in patients with well compensated cirrhosis and in those with surgical shunts (mainly portacaval anastomosis) compared with values found in cirrhotic patients with iron deficiency due to bleeding (from Williams *et al.*, 1967).

studies provide little evidence that the shunt affects iron absorption although increased hepatic iron deposition may occur as a result of changes in distribution (Doberneck *et al.*, 1963; Rubin *et al.*, 1964). The percentage of patients with an increased iron absorption after a portacaval shunt is similar to that found in non-operated cirrhotics (Fig. 6.8) and the development of siderosis is probably related to a continued increase in absorption with lessening of the amount of blood lost from varices.

### Bantu Siderosis

Some idea of the frequency with which this occurs can be obtained from the autopsy figures of Bothwell & Isaacson (1962) for the Johannesburg area. Only 30 per cent of the males examined had hepatic iron concentrations within the normal range and 37 per cent had severe siderosis with concentrations

comparable to those found in idiopathic hæmochromatosis. In women the incidence was lower only 12 per cent having severe siderosis.

The siderosis is undoubtedly due to a very high oral intake of iron resulting from the use of iron pots in the preparation of food and Kaffir beer. The average adult male Bantu consumes 50–100 mg of iron daily in beer alone. Over 80 per cent of this iron is present in an ionizable form and although the percentage absorption is decreased the net amount absorbed (2–3 mg daily) is more than sufficient to account for the degree of siderosis observed in middle life (Bothwell *et al.*, 1964).

Studies of the distribution of the iron deposits at autopsy show two distinct patterns. In the majority of cases iron is found in the reticuloendothelial cells of the spleen, bone marrow and lymph nodes and to a lesser extent in the parenchymal cells of the liver. In a small percentage of cases hepatic siderosis is severe and is associated with cirrhosis. These patients also have extrahepatic parenchymal deposits of iron. The development of such deposits may be related to the presence of cirrhosis and to a higher percentage saturation of the total iron-binding capacity (Bradlow, Dunn & Higginson, 1961). Clinically such cases are indistinguishable from idiopathic hæmochromatosis but pathologically there are a number of distinct differences. The concentration of iron in the spleen and in the portal tracts and Kupffer cells of the liver is higher in Bantu hæmochromatosis than in the idiopathic variety (Bothwell *et al.*, 1965).

Since the major source of iron is in alcoholic drinks the patients with the highest intake are likely to have consumed the greatest amount of alcohol and to have the highest incidence of malnutrition so that a number of factors may be concerned in the occasional development of cirrhosis.

The increased incidence of porphyria cutanea tarda, scurvy and vertebral collapse in the Bantu appears to be related to the presence of siderosis (Bothwell, 1964). The osteoporosis may be directly due to the excess iron intake for in the rat Manis & Schachter (1962) have shown that iron and calcium compete for active transport in the duodenum.

### **Chronic Refractory Anæmia**

Secondary hæmochromatosis was first described in patients with aplastic anæmia treated by multiple transfusions. True hæmochromatosis, however, as opposed to hæmosiderosis, is found more frequently in certain refractory types of anæmia with marked marrow hyperplasia. These have been termed the sideroblastic anæmias since the normoblasts show a characteristic perinuclear ring of large hæmosiderin granules in the cytoplasm. The basic defect is a block in the utilization of iron for hæmoglobin synthesis coupled with destruction of maturing erythroblasts within the marrow before they can be released into the peripheral circulation, so-called "ineffective erythropoiesis". Secondary hæmochromatosis may also occur occasionally in thalassæmia, another condition in which faulty hæmoglobin synthesis is associated with marrow hyperplasia. It is rare in pure hæmolytic anæmias.

This association of secondary hæmochromatosis with anæmias characterized by marked marrow hyperplasia and the observations that the amount of iron present at autopsy may be greater than that known to have been given by transfusion during life, led to the suggestion that the absorption of iron

from the gut was increased. Such iron may be deposited preferentially in hepatic parenchymal cells whereas iron released by hæmolysis or from transfused red cells is deposited in the reticulo-endothelial system of marrow and spleen where it is possibly less harmful (Williams & Pitcher, 1963).

Clinical measurements of absorption have been few. Losowsky & Hall (1965) in a study of a large family with a hereditary sideroblastic anæmia reported an increased absorption with increased deposition in the liver. Absorption, however, was increased in only one of 11 cases of sideroblastic anæmia of varying ætiology examined by Brain & Herdan (1965) and the concentration of iron in the liver but not in the marrow appeared to be directly related to the amount of therapeutic iron or blood transfusions given.

*Varieties of Sideroblastic Anæmia.* The many causes given in Table 6.VI all have in common an effect on the processes leading to the synthesis of hæmoglobin. In some the interference occurs early in the formation of porphyrin whilst in others the block appears to be in the final stage of iron incorporation into porphyrin. The hereditary form is rare. Transmission is recessive and sex-linked and only the males are severely affected (Losowsky & Hall, 1965).

TABLE 6.VI

CLASSIFICATION AND CAUSES OF A SIDEROBLASTIC ANÆMIA  
(Abridged from Morrow & Goldberg, 1965)

## 1. PRIMARY

- (a) Hereditary sex-linked
- (b) Acquired

## 2. SECONDARY

- (a) Associated with lead poisoning
- (b) Following antituberculous drugs—  
  INAH, cycloserine, pyrazinamide
- (c) Associated with:  
  Carcinoma, myeloma, leukæmia,  
  myeloproliferative disorders,  
  rheumatoid arthritis, poly-  
  arteritis nodosa.

The appearance of the sideroblasts is the same in the primary and secondary varieties (MacGibbon & Mollin, 1965), but some help in their differentiation may be obtained from the associated hæmatological changes. Hypochromia is relatively uncommon in secondary sideroblastic anæmia, megaloblastic change more frequent and the serum iron level and transferrin saturation more variable (Morrow & Goldberg, 1965).

**Treatment.** In many cases of the primary variety the disorder is mild and does not require treatment. In more severe cases pyridoxine therapy is worth a trial. The response is best in the hereditary form but the remission is rarely complete and large doses (100–1,000 mg) may be necessary to maintain improvement. It is important to look for associated deficiencies in folic acid, B<sub>12</sub> or ascorbic acid which may be aggravating the anæmia.

The question will arise as to whether attempts should be made to remove the excess iron. The intracellular accumulation of iron may inhibit several stages of hæm-synthesis. About 80 per cent of hæmsynthetase, an enzyme concerned in hæm-synthesis, is contained in the mitochondria (Lochhead &

Goldberg, 1961) and it is known that the characteristic hæmosiderin granules in the ring sideroblasts are formed by the accumulation of ferritin in mitochondria (Bessis & Jenson, 1965). In some cases iron has been removed successfully by frequent small venesections with accompanying improvement in the anæmia (Verloop, Bierenga & Diezeraad-Njoo, 1962).

It is to be stressed that oral iron therapy is not only unnecessary in this condition but may be positively dangerous. Indeed secondary hæmochromatosis has been reported in completely normal subjects who have taken large quantities of oral iron medicaments for many years (Turnberg, 1965).

### **Congenital Transferrin Deficiency**

This was first described by Heilmeyer and co-workers (1961) in a seven-year-old girl who presented with a refractory hypochromic anæmia. Anæmia had first been noted at the age of three months and both parents had a reduced transferrin level. At autopsy there was marked hæmosiderosis with cirrhosis and pancreatic fibrosis. It was suggested that as a result of the transferrin deficiency absorbed iron rapidly leaked out through the capillaries and inadequate amounts reached the marrow for hæmoglobin synthesis.

## **CHELATING AGENTS**

Although much enthusiasm followed the introduction of the effective iron chelating agents, diethylene triamine penta-acetic acid (DTPA) and desferrioxamine in the early 1960s, their clinical application has proved to be rather limited.

### **Diagnosis of Iron Overload**

The amount of iron excreted in the urine after a parenteral injection of DTPA or desferrioxamine is approximately proportional to the extent of the iron stores. Different workers use different doses and routes of administration. One scheme is to give 500 mg desferrioxamine intramuscularly (which unlike DTPA is not painful) and collect urine for six hours (Wöhler, 1964). Normal subjects excrete less than 0.5 mg, and patients with hæmochromatosis up to 5 mg.

Such tests may be of value in the diagnosis of affected relatives of patients with hæmochromatosis and in their follow-up to determine progression of the disorder (Walsh *et al.*, 1963; Powell, 1965). They can also be used to distinguish between alcoholic cirrhosis with a secondary mild siderosis and idiopathic hæmochromatosis (Walsh *et al.*, 1965). Their main limitation is that they give no information regarding the extent of the associated tissue damage.

The exact site at which chelation occurs is uncertain. *In vitro* studies have shown that desferrioxamine removes iron more easily from ferritin than from hæmosiderin. It can also remove iron from transferrin. A varying proportion of the iron in ferrioxamine formed *in vivo* after administration of desferrioxamine is re-utilized for hæmoglobin synthesis so that measurement of the amount of ferrioxamine or iron excreted in the urine underestimates the total amount of iron chelated. To allow for this Fielding (1965) gives a small dose of  $^{59}\text{Fe}$  labelled ferrioxamine at the same time as the parenteral dose of desferrioxamine. From the total amount of ferrioxamine excreted in the urine,

which is estimated chemically, and the proportion of the radioactive dose that appears there it is possible to calculate the total amount of ferrioxamine formed *in vivo* by chelation. Fielding found that total chelatable iron values were as high in hæmolytic states as in hæmochromatosis where the estimated iron stores were considerably greater. He suggested that there may be two sources of chelatable iron notably ferritin-hæmosiderin and iron from newly released hæmoglobin which is more readily chelatable.

### Treatment of Iron Overload

Both chelating agents have been used in treatment of primary hæmochromatosis. Although high values have been reported most workers find that the average amount excreted with daily parenteral injections varies between 10 and 30 mg. This has to be compared with the 200–250 mg of iron removed in a single venesection. There is also a tendency for excretion to diminish with repeated doses of chelating agent. It has been suggested that desferrioxamine should be given orally at the same time in order to chelate food iron and decrease the amount absorbed. Hwang & Brown (1965) have shown, however, that although desferrioxamine reduces absorption of ferrous salts it has no effect on the absorption of hæmoglobin iron or of iron in food.

Chelating agents may have a place in the treatment of secondary hæmochromatosis due to a refractory anæmia in which the hæmoglobin level is too low to allow therapy by small venesections. They have also been used in thalassæmia (Sephton-Smith, 1964) but in this condition as in other varieties of severe anæmia with siderosis the total amount of iron excreted may be less than that which continues to be added as a result of transfusions or increased absorption.

Desferrioxamine is of great value in the treatment of children accidentally poisoned by ferrous sulphate tablets (Jacobs, Greene & Gendel, 1965; Whitten *et al.*, 1965). After gastric lavage with bicarbonate solution desferrioxamine should be instilled into the stomach, suggested doses being 3,000–7,000 mg in 50–200 ml. of water or saline. This will prevent absorption of any iron still present in the gastro-intestinal tract. At the same time an intravenous drip should be set up and desferrioxamine infused in a maximum dose of 15 mg/kg/hour to a total of 80 mg/kg. Desferrioxamine is rapidly absorbed from muscle and it may be wise to give 2,000 mg i.m. before starting the time consuming gastric lavage and intravenous infusion.

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## CHAPTER 7

# CLINICAL PHARMACOGENETICS\*

by

DAVID A. PRICE EVANS

THE term pharmacogenetics was introduced into medical literature by Vogel (1959) and may be defined as the study of genetically determined variations that are initially revealed by the effects of drugs (see also: Kalow, 1965a; Motulsky, 1964). Genetically determined conditions in which symptoms are frequently spontaneous but may be precipitated by drugs—such as steroid-precipitated diabetes or barbiturate-precipitated porphyria—are not included.

It is a common and long established clinical observation that human beings show great variation in their responses to drugs (Williams, 1956).

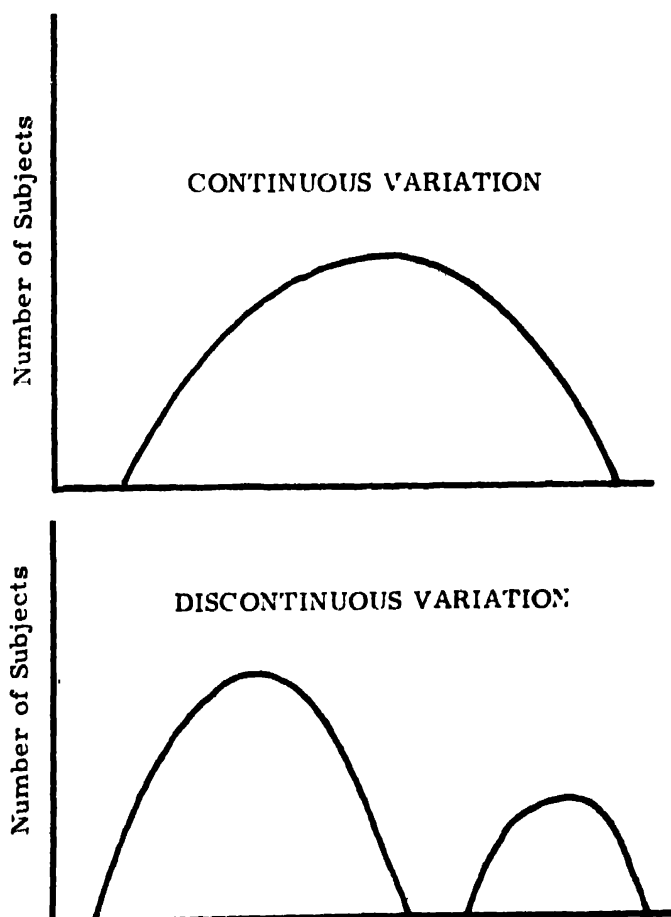


FIG. 7.1. The types of variability of frequency distributions which may be observed when large numbers of persons are given a standard dose of a drug in exactly the same manner (from Evans, 1962).

When a drug is administered experimentally to a large number of persons in strictly controlled conditions, their collective response may be either continuous or discontinuous. This is shown diagrammatically in Fig. 7.1. Continuous variation gives a unimodal curve often approximating to a normal distribution. Discontinuous variation on the other hand gives a bimodal or even a trimodal curve, the modes of which may be completely or incompletely separated from each other. In terms of clinical events, individuals represented in one distinct mode may show an apparently "all or none" phenomenon, often as a "side-effect" of the drug, e.g. apnoea in the case of suxamethonium. Thus although it has been more or less implicit in medical thought that variability of drug metabolism and response is of the unimodal continuous type, it has recently become apparent that there are examples of discontinuous variability which are of clinical interest.

Each mode in a system exhibiting discontinuous variability may represent a genetically determined phenotype (or a directly recognizable genotype), provided that artefact can be excluded as a causative factor. Such a hypothesis can be tested by the analysis of family data, and most of the remainder of this chapter deals with examples of this type of "polymorphic" system. Polymorphism implies the existence of several forms or phenotypes within the same population in the same environment at the same time, maintained from one generation to the next by genetic mechanisms. In Man the ABO blood group system is a typical example, in which phenotypes are distinguished by means of antisera. In the examples of polymorphism described here, the phenotypes (or genotypes) are detected by drugs.

In contrast to discontinuous distribution curves, the analysis of unimodal curves yields much less information concerning genetic factors, and the relatively few studies which have been performed in this area of human pharmacogenetics are considered in this chapter in a small separate section.

### GLUCOSE-6-PHOSPHATE DEHYDROGENASE

The degradation of glucose can be accomplished by two different mechanisms in animal cells. These are the Embden-Meyerhof and pentose monophosphate pathways (Fig. 7.2(a)). Although both pathways start with glucose and end with pyruvate, and although both pathways are to some extent reversible in some tissues, they subserve different roles in body economy. The Embden-Meyerhof pathway utilizes NAD (DPN) as a co-enzyme; movement of carbon atoms along this pathway from glucose to pyruvate causes the production of  $\text{NADH}_2$  (DPNH) and by this means accessory vital reactions are set in motion. The pentose monophosphate pathway, however, utilizes NADP (TPN) as a coenzyme. A key enzyme in the pentose monophosphate pathway is glucose-6-phosphate dehydrogenase (G6PD). Figure 7.2(b) shows how activity of this enzyme allows other reductions to proceed and it will be seen below that these reductions are in some way important in preventing erythrocytic hæmolysis.

#### Different Forms of G6PD

A number of different forms of G6PD have been discovered by electrophoresis, kinetic studies, pH optima, and substrate specificity determinations. This enzyme displays a number of polymorphisms.

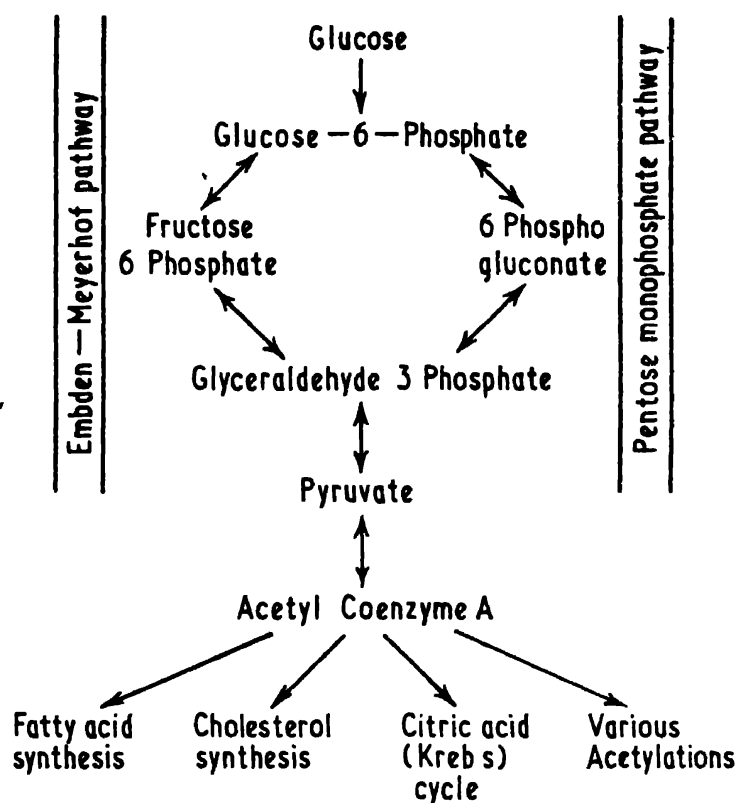


FIG. 7.2. (a) Outline schema of cellular carbohydrate metabolism. One arrow may represent one or several enzyme reactions.

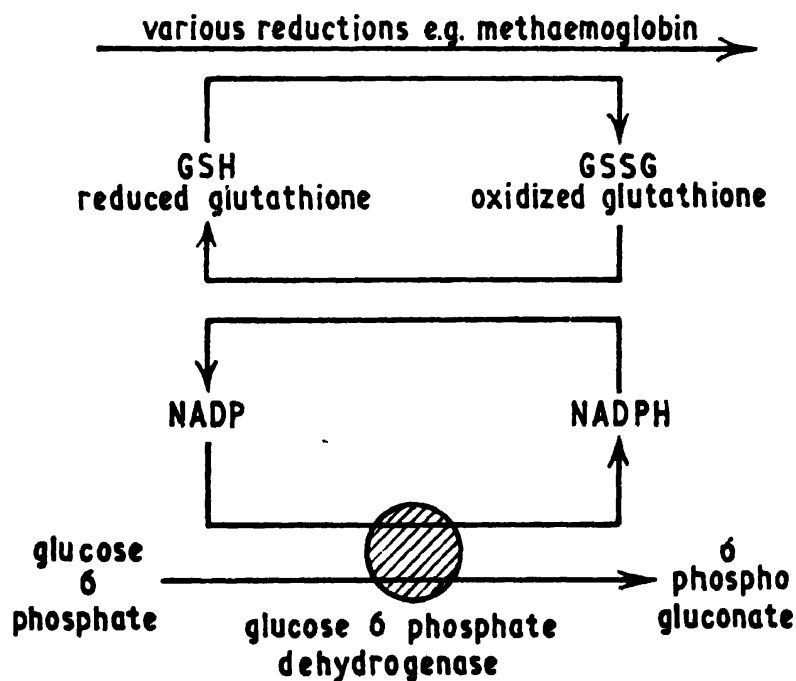


FIG. 7.2. (b) The site of the metabolic defect in primaquine sensitivity (indicated by the shaded area). (From Evans & Clarke, 1961.)

Boyer, Porter & Weilbacher (1962) demonstrated two electrophoretic variants of the enzyme in white blood cell homogenates from normal Negro subjects. Males show one of the two variants, A or B. Females show either A or B or sometimes A plus B. Similarly Porter and co-workers (1964) found two electrophoretically slow-moving variants of the enzyme (C or Baltimore, and Ibadan). These varieties can be associated with nearly normal activities and normal thermo-stability and kinetic characteristics.

American Negro subjects with deficient amounts of red cell G6PD have an electrophoretic mobility of the enzyme almost identical with type A. This variant is termed A<sup>-</sup> and subjects possessing it are healthy without chronic anæmia and reticulocytosis. The current view is that a deficient number of active enzyme molecules are produced in these subjects.

In Mediterranean G6PD deficient subjects the enzyme (unlike that of deficient Negroes) exhibits many functional peculiarities suggesting a different genetic variant. These peculiarities include a low Michaelis constant for glucose-6-phosphate, loss of substrate specificity and a bimodal pH optimum curve (Kirkman, McCurdy & Naiman, 1964).

Other atypical variants of G6PD which are probably rare have been described e.g. (1) G6PD "Seattle" in Caucasian (Welsh-Scottish) subjects who exhibit no hæmatological abnormality (Kirkman, Simon & Pickard, 1965) (2) in some cases of congenital non-spherocytic hæmolytic anæmia (see below).

## Genetics

### Family Studies of G6PD Deficiency

Direct methods of G6PD assay were not available until fairly recently. Before these methods became available the enzyme could be measured by means of the side reaction reducing glutathione shown in Fig. 7.2(b). This technique was employed by Childs *et al.* (1958) to elucidate the mode of inheritance of G6PD deficiency. They studied glutathione stability in an unselected Negro population and obtained bimodal frequency distribution curves. Men falling into the low mode of glutathione stability (i.e. those in whom relatively little reduced glutathione was present after incubation of their red cells with acetyl phenylhydrazine and glucose) were used to ascertain families which were then studied.

Pedigree analysis and the sex differences led to the conclusion that the gene controlling the "presence" or "absence" of glucose-6-phosphate dehydrogenase is on the X chromosome, i.e. the trait is a sex-linked dominant character, the penetrance of the gene being incomplete because daughters with intermediate glutathione stability and primaquine-sensitive sons are sometimes found to have apparently normal parents. The gene frequency figure in the American Negro population studied by Childs and co-workers (1958) is 0.14 (= 14 per cent = the frequency of affected males), and this gives a frequency of heterozygotes of 0.24 and affected homozygotes (females) of 0.02. This agrees well with the data obtained from a survey of 144 males and 184 females of the Negro race attending medical and pædiatric outpatient clinics, of whom 15 per cent of males and two per cent of females were in the sensitive group. This mode of inheritance has been confirmed

by Siniscalco and co-workers (1960) who studied 41 complete and 11 incomplete Sardinian families.

A family in which there was an X-linked marked elevation of erythrocytic G6PD activity has been described by Dern (1966). The G6PD in subjects possessing this trait has not yet been fully characterized biochemically. High G6PD activity was not associated with any clinical or hæmatological abnormality.

### Minor Genes Controlling G6PD

It is generally agreed that a single major gene controlling a polymorphic character functions against a background provided by a number of other genes. There may be alternative versions ("alleles") of these other genes also, and hence the background against which the major gene operates can vary between individuals. The background genes should vary less within families than between families and so siblings who are of the same character as determined by the major gene should be similar to each other, for example in enzyme activity values.

This idea has been tested out using G6PD by Davidson, Childs & Siniscalco (1964) by measuring erythrocyte G6PD activity in 32 pairs of normal (i.e. not G6PD deficient) male sibs with a heterozygote mother.

They found much greater variability between sibships than within sibships indicating a high intrafamilial homogeneity of erythrocyte G6PD activity and considerable interfamilial heterogeneity. The authors, therefore, suggest that the segregation of one allelic gene in a single family and multiple alleles within the population could account for these observations.

### Linkage Studies

The term linkage in genetics denotes topographical propinquity of genes on a chromosome so that they are separated less frequently than random in the process of "crossing-over".

It is known that the genes controlling both protan and deutan types of colour vision defect, those controlling the Xg blood group and those controlling G6PD activity are located with many others on the unpaired portion of the X chromosome. A possibility, therefore, exists that some of these genes are situated near enough to each other on the X chromosome to exhibit the phenomenon of linkage during transmission from one generation to the next.

The study of Siniscalco *et al.* (1960) revealed that a linkage existed between the glucose-6-phosphate dehydrogenase locus and the locus for red-green colour blindness. In an elegant study of eight Negro families in which there was segregation for abnormal colour vision and glucose-6-phosphate dehydrogenase deficiency, Porter, Schulze & McKusick (1962) showed a close linkage between the glucose-6-phosphate dehydrogenase locus and that for deutan\*

\* *Protanopia and Deutanopia* are two forms of red-green colour blindness. The terminology is based on the Young-Helmholtz theory of three primary colours. (Red—prot—first, green—deut—second, blue—tri—third.) Approximately two per cent of males have dichromatic vision and appreciate only two of the three primary colours. These are approximately equally divided between protanopia and deutanopia, tritanopia being very rare. Whilst the presence of dichromatic vision (as compared with the normal trichromatic) can be detected using simple means such as Ishihara charts, the differentiation of protanopia from deutanopia requires laboratory examinations which are outside the usual clinical sphere, for example response to the various wavelengths of the visible spectrum (see Sorsby, 1964).

colour vision, but not between the glucose-6-phosphate dehydrogenase locus and that for protan colour vision. Siniscalco, Filippi & Latte (1964) produced further evidence showing that the G6PD locus must be located between the deutan and protan loci; though it seems somewhat closer to the deutan locus. Despite earlier reports suggesting the presence of a linkage between the Xg\* blood group and the G6PD loci, very extensive recent studies have shown that these two loci are not within practicable measurable distance of each other (Adam *et al.*, 1967; Siniscalco *et al.*, 1966).

### The Lyon Hypothesis

The Lyon hypothesis explains the similarity of expression of genes on the unpaired segment of the X chromosome in men and women by proposing that in each somatic cell of the female, one of the two X chromosomes is genetically inactive. The inactivation must occur early in development, and it is a matter of chance whether the maternal or paternal X is inactivated. Once an X chromosome is inactivated in a developing cell, all progeny of that cell presumably maintain the same inactive X. This hypothesis has been very neatly put to the test by Davidson, Nitowsky & Childs (1963) by examining single cells in a Caucasian female heterozygous for G6PD. Clones derived from single skin cells possessed quantitative G6PD activity either in the deficient range or in the normal range. Similarly in a qualitative study, clones derived from single skin cells of Negro females heterozygous for G6PD were examined electrophoretically. These women possessed both the A and B bands referred to above, when typed on a number of cells taken straight from the skin, but the clones grown from single skin cells possessed either A or B mobilities but not both.

Hence as far as the human G6PD locus is concerned, the Lyon hypothesis seems to hold true.

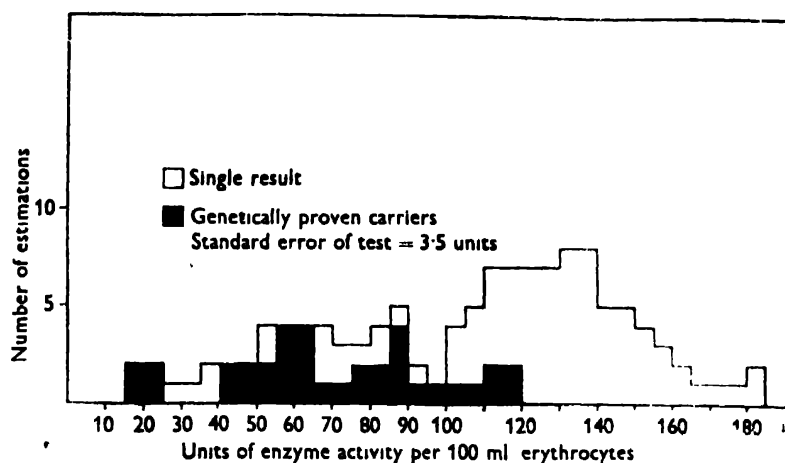
### Detection of G6PD Deficiency

G6PD activity can be estimated directly by the change in absorbancy (optical density) produced by the reduction of NADP (Fig. 7.3). A bimodal distribution is obtained in a population, the lower mode representing a low level of activity rather than complete absence. The bimodality is much more clearly defined in men than in women and there is notable difficulty in identifying heterozygous Negro women.

For field work on rural populations very accurate results may not be required and the use of complicated apparatus may not be practicable. Simple tests utilizing dye decolorization and methæmoglobin reduction are available for such surveys.

Motulsky's brilliant cresyl blue decolorization time test enables a frequency distribution histogram of a population to be constructed which shows persons deficient in G6PD forming a small mode with a long decolorization time. Similarly the ability to reduce methæmoglobin in the presence

\* *Xg Blood Group*. A blood group system which is sex-linked, i.e. the genes controlling the characters are on the X chromosome. In the original series tested 61.7 per cent of men were Xg (a+) whilst 38.3 per cent were Xg (a-). For women the percentages were Xg (a+) 88.8 and Xg (a-) 11.2 (Race and Sanger, 1962).



(a) Distribution in 70 Negro women.

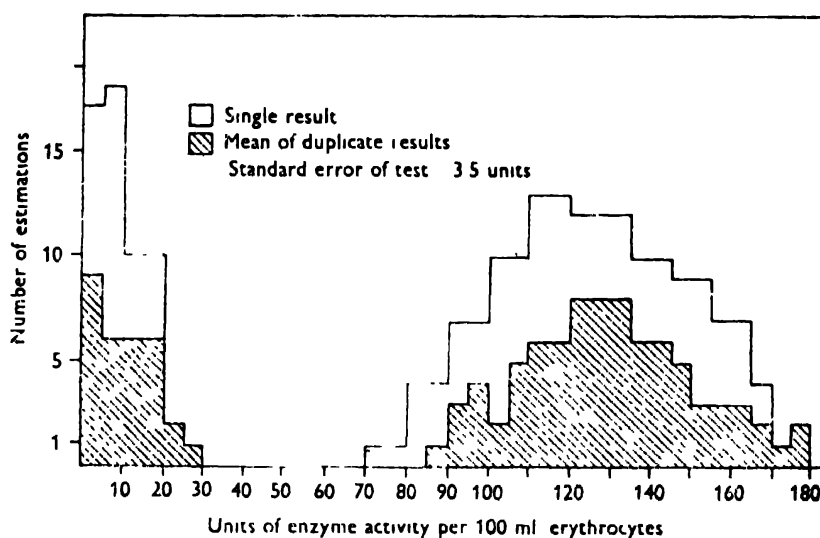
(b) Distribution in 114 Negro men (from Porter *et al.*, 1962).

FIG. 7.3. Glucose-6-phosphate dehydrogenase activity values.

of methylene blue is much less in G6PD deficient cells than in normal red cells. This test can be used with direct vision or with instruments.

### Sensitivity to Primaquine and Other Drugs

The development of hæmolytic anæmia by a minority of subjects receiving the 8-amino-quinoline drugs used since 1926 in the eradication of exo-erythrocytic forms of *Plasmodium vivax* malaria in Man was the clinical observation which resulted in the discovery of the genetic mechanism responsible for the control of glucose-6-phosphate dehydrogenase production.

Although a few early studies of this hæmolytic anæmia were made, it was the wide-scale introduction of primaquine during the Second World War that led to the intensive study of this phenomenon.



When a primaquine-sensitive person is given 30 mg daily there is little or no evidence of hæmolysis for two or three days, but eventually the urine becomes dark, and pain in the loins and jaundice appear. The hæmoglobin concentration of the blood falls, associated with the development of Heinz bodies (denatured protein) in many of the red blood cells, and the Coombs' test is characteristically negative. In relatively mild cases, these phenomena regress and the patient feels well, even if the administration of the drug is continued, and a temporary reticulocytosis accompanies the increase in the hæmoglobin concentration of the blood.

Studies using radio-isotopes provided an explanation for this recovery. Dern and co-workers (1954) transferred  $^{51}\text{Cr}$ -labelled erythrocytes from a sensitive subject into non-sensitive recipients. The cells survived normally until primaquine was administered, at which time rapid hæmolysis occurred. In the converse experiment, transferred normal cells withstood hæmolysis when primaquine was administered to a primaquine-sensitive subject even when his own cells underwent lysis. Further studies with  $^{59}\text{Fe}$ -labelled primaquine-sensitive erythrocytes of known age revealed that upon ingestion of primaquine these cells lysed only when they were at least 63 to 76 days old. Red cells 8 to 21 days old did not lyse. This observation provided an explanation for the clinical phenomenon already described. When a subject continues to take the drug, the mean age of his erythrocyte population falls until the predominantly young cells which remain are capable of withstanding the hæmolytic effect of the drug.

Glucose-6-phosphate dehydrogenase is reduced in activity in primaquine-sensitive subjects. This discovery followed the observation that when sensitive and non-sensitive cells were incubated *in vitro* with primaquine in glucose, the reduced glutathione content fell only in the sensitive cells and not in the non-sensitive cells, while in the absence of glucose the reduced glutathione content of both sensitive *and* normal cells fell. The fall in reduced glutathione content in the sensitive cells was thus thought to result from an impaired ability to metabolize glucose, biochemically most likely to be due to a defect in dehydrogenation of glucose-6-phosphate (Fig. 7.2(b)). This deficiency is more marked in older cells.

Negro G6PD deficient subjects also experience more hæmolysis than normal subjects when treated with 4-4'diamonidiphenyl sulfone (syn DDS, Dapsone).

The relative susceptibility of Caucasians with G6PD deficiency to drugs other than primaquine has not been quantitated (Carson & Frischer, 1966) but it seems clear that Mediterranean G6PD deficient subjects are sensitive to a very much wider range of drugs than their Negro counterparts (Tarlov *et al.*, 1962), including certain sulphonamides, phenacetin, acetanilide, furandantin, antipyrin, probenecid, para-aminosalicylic acid and acetyl salicylic acid. They are also liable to develop a severe hæmolytic anæmia as a result of eating the broad bean *Vicia faba*, or even by inhaling the pollen of the same plant.

#### **Congenital Non-Spherocytic Hæmolytic Anæmia**

Selwyn & Dacie (1954) classified congenital non-spherocytic hæmolytic anæmia (CNSHA) into two types, as a result of hæmatological studies.

The majority of patients belong to Type I, characterized by icterus and splenomegaly, males being affected more frequently than females, and they have round to elliptical erythrocytes with minor degrees of anisocytosis and poikilocytosis. The osmotic fragility of fresh blood is normal, but there is a tendency to abnormally increased resistance after incubation; autohæmolysis rates are within the normal range or only slightly increased. Hæmoglobin values in Type I range from 6.3 to 13.1 g per 100 ml and reticulocyte counts 3.7 to 23 per cent. (In type II hereditary non-spherocytic hæmolytic anæmia, on the other hand, autohæmolysis rates are considerably elevated.)

Patients with Type I disorder are now known to have abnormalities of G6PD, but there appears to be considerable variability between them. Some of these patients have less than two per cent of the normal activity of the enzyme in their red cells, others have G6PD which seems normal except for very rapid loss of stability during storage or heating, and yet other subjects had an unusual pH optimum curve (Kirkman *et al.*, 1964).

It is certain that further specific enzyme abnormalities will be found to account for other genetic entities in both types of CNSHA, and the usefulness of the division into Types I and II will then be at an end.

### Neonatal jaundice

It has been shown that severe neonatal jaundice may be associated with glucose-6-phosphate dehydrogenase deficiency in infants (Weatherall, 1960). Doxiadis, Fessas & Valaes (1961) pointed out that in Greece there is a high proportion of patients with severe neonatal jaundice which cannot be explained by either Rh or ABO incompatibility; moreover it has been shown that the jaundice is not due to an hæmoglobinopathy or inadequate conjugation of bilirubin. However, the same workers (Fessas, Doxiadis & Valaes, 1962) have shown that although neonatal jaundice is more common among infants with glucose-6-phosphate dehydrogenase deficiency than in those who are not deficient, severe neonatal jaundice is still not common. Nevertheless, when 81 families of infants with glucose-6-phosphate dehydrogenase deficiency and severe neonatal jaundice were investigated it was found that the deficiency was present in more than one child in 54 of these families. Severe neonatal jaundice had occurred in 11 male and 5 female siblings (all five of the male siblings tested were deficient in glucose-6-phosphate dehydrogenase). No jaundice occurred in 39 male siblings and 29 female siblings (of these male siblings 27 were tested and 10 were deficient in G6PD).

These investigators suggest that there is a second genetic factor which accounts for the development of severe neonatal jaundice in association with glucose-6-phosphate dehydrogenase deficiency in some families; they point out that this would account for the absence of severe neonatal jaundice associated with G6PD deficiency in some racial groups.

The nature of the stress eliciting hæmolysis is at present unknown. Some cases have been associated with naphthalene inhalation and others with the administration of vitamin K analogues, but these were only a minority of the cases studied by Doxiadis & Valaes (1964). A family history of either favism or drug hæmolysis should alert the pædiatrician to anticipate the occurrence of kernicterus.

This problem exists in the Far East as well as in the Mediterranean

basin, as the early paper of Weatherall (1960) and the recent paper of Yue & Strickland (1965) testify. These latter workers observed 9 severe cases of neonatal jaundice in 1,177 consecutive births, and 4 of these 9 were ascribable to G6PD deficiency. Neonatal jaundice also occurs in association with the relatively mild form of Negro G6PD deficiency (Ifekwunigwe & Luzzatto, 1966).

### Malaria

Whilst the prevalence in Great Britain of G6PD deficiency is low (0.9 per cent in South Wales according to Saunders, 1966) a high incidence of the defect (ten per cent or more) has been reported among American Negro, African, various Mediterranean, Iraqi, Persian, Indian, Indonesian, Thai and Formosan subjects, while lower incidences occur in populations of adjacent areas. The reasons for the existence of this polymorphism are unknown. Motulsky (1961) suggested that glucose-6-phosphate dehydrogenase deficiency may be in some way less favourable to the proliferation of malaria plasmodia. Motulsky (1964) shows a very striking correlation between G6PD deficiency and sickling trait in various populations.

In support of this, Allison & Clyde (1961) found that parasite rates and densities of *Plasmodium falciparum* were significantly lower in young male Tanganyikan children with erythrocyte glucose-6-phosphate dehydrogenase deficiency than in male children with normal enzymic activity. Although Wilson (1961) cast doubt on the deductions drawn by these investigators, Harris & Gilles (1961), studying Nigerian children four months to three years of age, found a lower mean  $\log_{10}$  *P. falciparum* ring count in children with glucose-6-phosphate dehydrogenase deficiency than in those with normal enzyme activity. It is to be noted that Allison & Clyde (1961) and Harris & Gilles (1961) studied untreated populations.

### Favism

As mentioned above, Mediterranean subjects with the severe form of G6PD deficiency are susceptible to severe hæmolysis not only following drug administration, but also following the ingestion of the bean *Vicia fava*. This condition, known as favism, has been known for many centuries in Mediterranean countries and has a familial incidence. Although G6PD deficiency is essential, some other factor in addition to G6PD deficiency may be required to produce the hæmolytic response to the bean. No such factor has yet been recognized (Tarlov *et al.*, 1962). Ideo and co-workers (1965a) showed the the lymphocyte G6PD activity is absent in subjects prone to favism, and similarly Ideo and co-workers (1965b) have shown that the granulocytes of favism subjects have only one-fifth of the normal G6PD activity.

### Moth Balls

A similar hæmolytic anæmia associated with abnormal reduced glutathione stability levels has been observed in some subjects following the ingestion of naphthalene moth balls (Zinkham & Childs, 1958). This may occur in children and in adults as a perversion of appetite (e.g. during pregnancy).

### THE ACETYLATION POLYMORPHISM

Isoniazid (1-isonicotinylhydrazide) was first synthesized by Meyer & Mally in 1912, but its chemotherapeutic value was not discovered until 40 years later. Grunberg & Schnitzer (1952) and Grunberg and co-workers (1952) showed that the compound had bacteriostatic action against *Mycobacterium tuberculosis* strain H37Rv *in vitro* and that it protected mice from tuberculosis when they were given intravenous injections of tubercle bacilli. Robitzek, Selikoff & Ornstein (1952) then showed that the compound was effective in the treatment of tuberculosis in Man.

A considerable amount of work has been carried out on the metabolism of isoniazid both in animals and in Man. These studies have shown that there is rapid and complete absorption of the drug, giving an initial high blood level. When this falls the greater part of the isoniazid appears in the urine, either unchanged or biotransformed, within 24 hours of oral administration (Elmendorf *et al.*, 1952; Barclay *et al.*, 1953).

A large variation in the metabolism of isoniazid was found to exist among human beings by Bönicke & Reif (1953), and Hughes and co-workers (1955). The latter workers studied the products excreted in the urine after isoniazid was given to human beings, and they found that (1) all the drug given could be accounted for; (2) isoniazid was found in the urine as free unchanged drug, acetyl isoniazid, isonicotinic acid and small quantities of other metabolic products; (3) there was an inverse relationship between the percentage of the given dose which is recovered as free drug and that recovered as acetylated derivative, the percentage recovered as acetyl isoniazid varying 14 to 70 per cent of the dose given; and (4) the pattern of excretion for a given subject remained constant even when the drug was given daily for months.

Distribution histograms of the percentage of the administered isoniazid which is excreted in the free unchanged form in the urine were given by Biehl (1957). These were bimodal, suggesting that subjects belong to one of two classes—either that of rapid or of slow inactivators.

#### Genetics

A twin study was carried out by Bönicke & Lisboa (1957) in which they estimated the free isoniazid excreted in the urine during 24 hours as a percentage of the dose administered. The results show that there is a remarkable similarity between monozygous twins and a considerable difference between dizygous twins.

Mitchell and co-workers (1958) also thought that this polymorphism for isoniazid metabolism in Man might be genetically determined. Support for this view was afforded by the finding that the proportion of rapid inactivators in groups of Japanese subjects was much larger than in groups of Caucasian subjects (Harris, Knight & Selin, 1958). To test the genetic hypothesis a study of 20 families was carried out by Knight, Selin and Harris (1959). They showed that it was probable that slow inactivators of isoniazid who also have high blood levels of the free compound and excrete a high proportion of the free drug in the urine had a trait recessive to rapid inactivation and moreover that the genes concerned were autosomal.

Evans, Manley & McKusick (1960) determined plasma isoniazid concen-

trations six hours after subjects had swallowed a dose of 10 mg isoniazid per kg body weight. The distribution histogram of these values is bimodal, allowing the phenotype of a subject to be expressed as either a rapid inactivator with a low plasma isoniazid concentration or a slow inactivator of the drug (Fig. 7.4). In this study 267 members of 53 Caucasian two-generation family units were phenotyped. Examination of the pedigrees proved that slow inactivation of isoniazid is an autosomal recessive trait. In addition, the mean six-hour plasma isoniazid concentration in recognizable heterozygotes is higher than that for other rapid inactivators in the pedigrees. This indicates that dominant homozygotes have lower values than the heterozygotes—in other words, a dosage effect is shown for the character (Evans *et al.*, 1960).

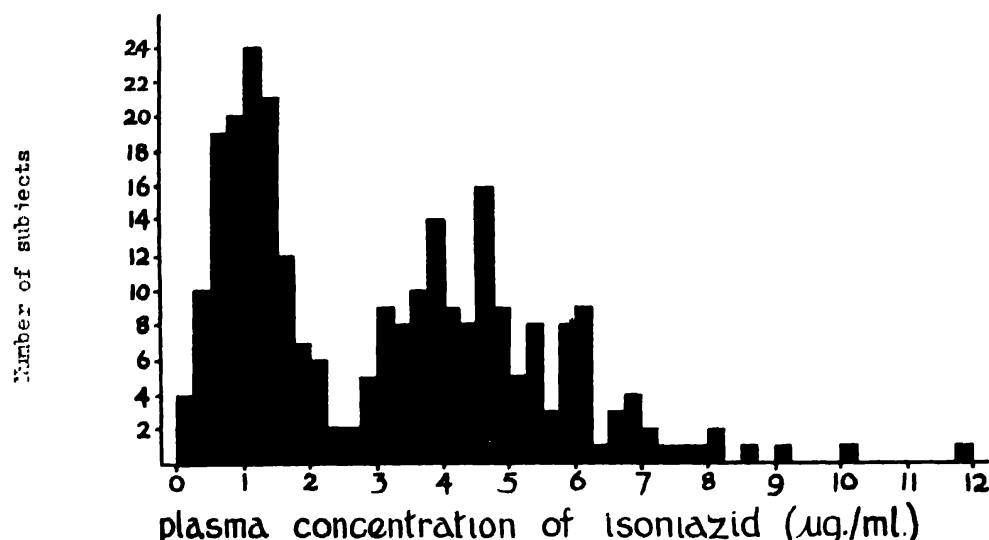


FIG. 7.4. Plasma concentration 6 hours following oral administration of 9.7 mg isoniazid per kg body weight in 267 members of 53 families (from Evans *et al.*, 1960).

The result of further work using a precise microbiological technique is that the genotype of a person can be determined directly from a trimodal distribution curve (Sunahara *et al.*, 1963a; Dufour, Knight & Harris, 1964). (See below.)

#### Biochemical Basis for the Polymorphism

Intestinal absorption factors are not responsible for the difference between the two phenotypes. Jenne, MacDonald & Mendoza (1961) have shown that the rapid and slow inactivator phenotypes are recognizable when isoniazid is given intravenously and the half-life of the plasma isoniazid concentration determined. The same investigators have shown that protein binding, renal glomerular clearance and renal tubular reabsorption of the drug are the same in both phenotypes. These facts strongly suggested that a metabolic difference existed between the two phenotypes, and in view of the observations of Hughes *et al.* (1955) mentioned above, it was likely that this difference consisted of different speeds of acetylation.

Evans (1962) has produced evidence to support this hypothesis. The acetylation of sulphadimidine in human volunteer subjects is polymorphic;

rapid inactivators of isoniazid being extensive acetylators of sulphadimidine. When supplied with ATP, acetate and coenzyme A, biopsy specimens of liver from rapid inactivators of isoniazid have greater acetylating powers for isoniazid than those obtained from slow inactivators. One enzymic step, namely that catalysed by acetyl-transferase, was shown to be polymorphic by incubating healthy liver biopsy specimens with sulphadimidine and acetyl Coenzyme A (Evans & White, 1964).

Peters, Gordon & Brown (1965) confirmed that sulphadimidine (syn: sulphamethazine) and isoniazid were both polymorphically acetylated in the same way, and by careful analytical techniques Peters, Miller & Brown (1965) showed that acetylation was the primary metabolic reaction determining inactivator status for isoniazid.

Jenne (1963) found that jejunal mucosa as well as liver homogenates showed *in vitro* polymorphic capacity to acetylate isoniazid. Jenne (1965) partially purified and concentrated the acetyl-transferase 300-fold. From livers representative of the "rapid" and "slow" groups concentrated preparations of the enzyme showed no differences in apparent Michaelis constant for isoniazid and in several other respects the enzymes behaved identically. The difference in activity of these groups do not appear to be on the basis of differences in affinity of the enzyme for isoniazid, but may be due to differences in amount of an identical enzyme molecule.

The enzyme acetylating isoniazid in human liver has been shown to be "non-inducible" (see section below) by Levi & Walker (1966), and it is in keeping with this that Sunahara, Urano & Kawait (1963b) showed that acetylating enzymes in animals were mostly located in liver cell supernatant fractions.

Skin and white blood cells have been shown to be devoid of the capacity to acetylate sulphadimidine *in vitro* (Evans & White—unpublished observations). Red cells similarly lack the capacity to acetylate isoniazid (Motulsky & Steinmann, 1962).

#### **Other Drugs for which there is Evidence for Polymorphic Acetylation**

Evidence has been obtained to show that hydrallazine is most probably a substrate for polymorphic acetylation in Man. Evans & White (1964) showed by *in vitro* incubation of liver homogenates containing acetyl coenzyme A and hydrallazine that more unconjugated drug was present at the end of incubation in slow than in rapid acetylator livers. Jenne (1965) showed competitive inhibition by hydrallazine of isoniazid acetylation by partially purified liver preparations. McIsaac & Kanda (1964) had demonstrated in rabbits that 25 to 30 per cent of [ $^{14}\text{C}$ ]-1-hydrazinophthalazine given either intragastrically or intraperitoneally appeared in the urine as N-acetyl-1-hydrazinophthalazine.

It is probable that some other sulphonamides as well as sulphadimidine will undergo polymorphic acetylation in Man. Suggestive evidence for this process was obtained during pharmacological studies on the combination tablet "Bimez" at the ICI laboratories some years ago. Persons who acetylate sulphadimidine to a considerable extent also deal with sulphamaprine in the combination tablet in a similar way (Spinks, 1962, personal communication). White & Evans (1967) showed that the percentage of urinary sulfamethoxy-

pyridazine acetylated was greater in rapid than in slow acetylators; but that the polymorphism had no significant effect on the serum concentrations of the free (non-acetylated) drug.

### **Toxicological Aspects**

Early studies by Hughes and co-workers (1954) and Biehl (1957) had suggested that slow inactivators of isoniazid were more susceptible to develop peripheral neuropathy than rapid inactivators of the drug. This was amply confirmed by Devadatta and co-workers (1960) in a much bigger survey in Madras.

It is probable that the acetylation polymorphism also underlies the observation of Smirnov & Kozulitzina (1962) that toxic effects of the hydrazide drug phtivazid were noted relatively seldom in patients excreting large amounts of the drug in the acetylated form.

The very bizarre toxic effects of hydrallazine which include a systemic lupus erythematosus-like syndrome and peripheral neuropathy (see Meyler, 1960) may well be more common in slow acetylators of the drug, but the relative infrequency of suitable subjects precludes the easy experimental verification of the hypothesis.

Phenelzine, the antidepressive drug (Nardil), which is a substituted hydrazine, should from its molecular configuration be a substrate for polymorphic acetylation. Evans, Davison & Pratt (1965) in a double-blind investigation found that the severe side-effects of phenelzine occurred only in slow acetylator subjects.

### **Observations on Possible Natural Substrates**

The natural substrate(s) for the human acetylation polymorphism remain(s) unknown. Peters, Miller & Brown (1965) estimated values of dimethyl-amino-benzaldehyde positive substances in urines of persons who had not taken drugs. These values were estimated after hydrolysis, and so should include all hydrazines and gave a value below 0.8 mg per 12 hours. This would suggest that naturally occurring hydrazines are unlikely to be of much importance.

Hexosamine and tryptophan metabolites are extensively acetylated by humans, but the available evidence does not suggest that they are substrates which are polymorphically acetylated (White & Evans—unpublished data).

### **Substances which are Monomorphically Acetylated**

The likelihood that para-aminosalicylic acid (PAS) and para-aminobenzoic acid (PABA) are monomorphically acetylated in human populations is suggested by the following observations.

Jenne and co-workers (1961) found that the fall-off of serum PAS concentrations was the same in rapid and slow acetylator subjects. Motulsky & Steinmann (1962) found that the acetylation of PAS by red cell homogenates was unimodally distributed amongst 131 subjects. Evans (1963) gave PAS orally to 52 healthy subjects and showed that the ratio of free to total drug (both determined chemically) in the urine was a unimodally distributed character. Evans (1964) showed that the PABA acetylating properties of human jejunal mucosa *in vitro* were not correlated with the power of liver

biopsy tissue from the same persons to acetylate sulphadimidine. Finally, Jenne (1965) gave 300 mg doses of PAS, separated urinary PAS and acetylated PAS chromatographically and showed that the ratio of the latter to the former was not different in rapid and slow acetylators of isoniazid.

Evans & White (1964) found that liver biopsy specimens when homogenized with acetate, ATP and coenzyme A would acetylate isoniazid and sulphadimidine but not sulphanilamide. In contrast, rat liver which was used in preliminary work was able to acetylate sulphanilamide in the same system. This observation was confirmed by Hartiala & Terho (1965) who showed that many rat and guinea pig tissues were able to acetylate sulphanilamide. Peters and co-workers (1965a), estimating the acetylation of excreted sulphanilamide, sulphadimidine and isoniazid in the same human subjects, demonstrated that the acetylation of sulphanilamide was closely similar in both slow and rapid acetylators of the other two drugs.

The evidence therefore points to the existence of two acetylating systems in Man—one polymorphic and the other monomorphic. Although it seems likely that the molecular electronics (Perault & Pullman, 1963) of substrates determine by which system they are dealt with in the body, this is an area in which comparatively little work has been done.

#### **Acetylator Phenotypes and Susceptibility to Disease Processes**

Since rapid acetylators have lower plasma isoniazid levels than slow acetylators, it might be expected that tuberculous rapid acetylators might respond less well to treatment.

This idea has been very thoroughly tested by Harris (1961) who studied 744 patients with pulmonary tuberculosis on standardized treatment schedules. There was a tendency for cavity closure and sputum conversion (i.e. disappearance of tubercle bacilli) to occur earlier in slow acetylators but the eventual outcome after six months treatment was the same in both phenotypes.

Gow & Evans (1964) similarly found that neither acetylator phenotype was associated with reversion (i.e. reappearance of tubercle bacilli in the urine) following a course of therapy for genitourinary tuberculosis.

As anticipated by McDermott (1960) the acetylator polymorphism does not seem to influence the outcome of tuberculosis treated by the standard schedules including isoniazid. The polymorphism may however turn out to be quite important where smaller or intermittent dosages are employed.

There is no association between the acetylator phenotype and the development of isoniazid resistant tubercle bacilli (Biehl, 1957; Harris, 1961).

Pantothenate deficient rats are unable to synthesize coenzyme A and hence suffer from an inability to acetylate thus simulating the metabolic situation in human slow acetylators. The reports of Berg (1959) and Zucker, Seronde & Zucker (1955) that pantothenate deficient rats develop duodenal ulcers led to speculation concerning possible association between human acetylator phenotype and duodenal ulcer. Evans & Kemp (1967) therefore subjected 56 patients with typical uncomplicated duodenal ulcers to a sulfanethazine test; and of these 27 were rapid acetylators (Fig. 7.5). There is thus no overwhelming susceptibility to duodenal ulcer in slow acetylators since



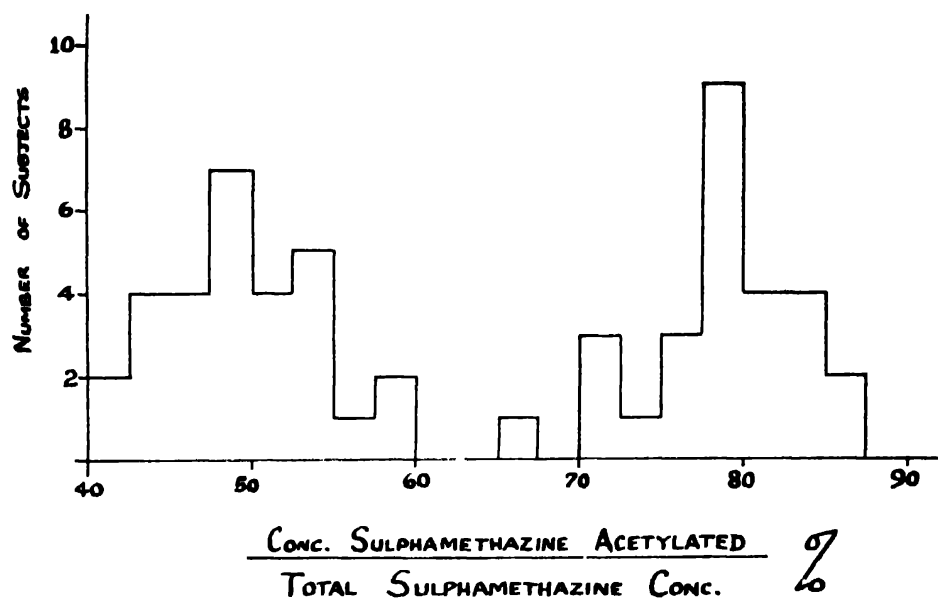


FIG. 7.5. Sulphamethazine acetylation in subjects with duodenal ulcers.

the phenotype incidences in this disorder are very similar to those found in healthy persons in the same population.

#### Racial Distribution of Acetylator Phenotypes

Using the precise microbiological technique referred to earlier, the serum isoniazid concentration can be very accurately determined. This method when applied to serum six hours after an oral dose of 4 mg isoniazid per kg body weight allows genotypes to be directly determined from a trimodal frequency distribution curve. Caucasian, Negro and Japanese populations have been studied in the same laboratory in this way by Dufour and co-workers (1964). In Negro and Caucasian populations the "slow" allele is approximately three times more frequent than the rapid allele, but in Japanese exactly the reverse proportions are found.

Impressive evidence for a "cline" of increasing frequency of the slow alleles from the Ainu in the North to the Thai in the South has been produced by Sunahara and co-workers (1963a) by studying populations along the coast of Asia and in the islands of Japan. This fits with an earlier observation by Armstrong & Peart (1960) that Eskimo populations contain only a very low percentage (about five per cent) of slow inactivators of isoniazid.

The implication of this work would seem to be that there is some connection between the "set" of the polymorphism and latitude—more rapid acetylators towards the Arctic circle and more slow acetylators near the Equator. This simple hypothesis is not sustained, however, by the similarity in acetylator phenotype distributions between the populations of Northern Europe and Africa. Meaningful ecological interpretations must probably await the recognition of the natural substrate.

#### DIPHENYLHYDANTOIN TOXICITY

The early signs of diphenylhydantoin toxicity are nystagmus ataxia and mental blunting and it seems that most people will develop these signs

when they consume 600 mg daily on a long term basis. Liver disease renders subjects more prone to develop these toxic signs but sometimes, however, these signs appear for no apparent reason in healthy individuals taking small doses of the drug.

Kutt *et al.* (1964) have investigated this phenomenon which occurred in an otherwise healthy 24 year old man who had been given 300 mg diphenylhydantoin daily to control seizures which developed after a head injury.

When this patient consumed 3.8 mg drug per kg body weight over a period of two weeks the plasma level of the drug rose progressively to approximately 70  $\mu\text{g}$  per ml.; and chromatographic studies on the plasma showed that the material which was measured in this way was almost all non-hydroxylated drug. Hydroxylated derivatives excreted in the urine accounted for 48 per cent of a daily dosage of 300 mg of drug.

Normal individuals consuming 4 mg drug per kg body weight showed a blood level stabilizing over two weeks to the range of 4 to 8  $\mu\text{g}$  per ml. Normal individuals excreted 60 to 70 per cent of this dosage of the drug as hydroxylated derivatives.

The patient's mother and brother showed a similar relative inability to para-hydroxylate diphenylhydantoin and developed toxic signs whilst consuming a daily dose of 300 mg.

It would appear that the hydroxylation inability in these genetic phenotypes is confined to the molecular structure of diphenylhydantoin. The degree of hydroxylation of phenobarbital and phenyl-alanine were the same in them as in persons who are normally able to hydroxylate diphenylhydantoin and the propositus did not develop barbiturate toxicity when he was treated with customary doses.

This family constitutes another example of genetically determined inability to metabolize a drug rendering patients particularly prone to develop toxic effects following its administration.

## VARIABILITY IN METABOLISM AND RESPONSE TO ANALGESICS

### Phenacetin, Salicylate and the Renal Tubules

A most interesting observation concerning the effect of phenacetin on the kidney has been made by Prescott (1965), using a staining technique whereby renal tubular cells, leucocytes and red blood cells can be differentiated and counted in urine samples collected over timed periods. Urine cell counts were made in five healthy males and five healthy females for five days to establish a base-line, and then for five days during which they ingested 3.6 g phenacetin daily. Two of the ten subjects responded with a considerable increase in renal tubular cell output, and one of these two subjects also produced a large increase in leucocyte output and a slight increase in red blood cell output. Prescott (1966) has now extended his observations to 27 healthy volunteer subjects and has found four high cell count "responders" amongst them. There was no evidence of occult chronic pyelonephritis in any of these volunteer subjects. The presence or absence of a response in tubular cell excretion is consistent in the same individual when the test is repeated. The "responders" did not produce an increased tubular cell excretion when they were given

*N-acetyl-p-amino-phenol*, which is a prominent metabolite of phenacetin. "Responders" and "non-responders" have closely similar plasma phenacetin half-lives (Prescott, 1966). It seems possible that these findings may point to a genetically determined polymorphic response of the renal tubule to phenacetin, but at present no family studies have been carried out to test this hypothesis.

Prescott (1965) also studied the effect of acetylsalicylic acid on renal tubular cell excretion using the technique outlined above. All subjects tested responded with a very great elevation of tubular cell output, though there was considerable variation between individuals. The possibility of a correlation between gastric cell exfoliation and renal tubular cell excretion in response to aspirin within individuals has hitherto not been investigated.

An analogy may be drawn between the renal tubular cell shedding and hæmolytic phenomena, similar to that suggested by Creamer (1964) between hæmolytic phenomena and intestinal cell shedding. There is reason to believe that the older cells in the tubule are shed in response to aspirin. Continued administration results in a "levelling off" of the shedding process as the epithelium settles down to a new equilibrium. This is comparable to the effect of an hæmolytic agent such as phenylhydrazine on the blood. The influence of phenacetin on the renal tubule may be more directly comparable to the influence of primaquine on the blood. Some persons—namely those who are G6PD deficient—are sensitive to its effect, whereas non-deficients do not respond with hæmolysis when ordinary clinical dosages are administered. Similarly phenacetin "responders" may have some enzymic defect in their renal tubular cell which causes them when effete to be shed readily through the influence of phenacetin.

The relationship of renal tubular epithelial shedding to the clinical problem of analgesic nephropathy remains obscure.

#### Salicylate Metabolism

Evans & Clarke (1961) published a frequency distribution histogram showing the serum salicylate concentrations in 100 subjects following oral drug administration. The wide inter-subject variability could clearly be due to a number of components, viz: variability of absorption, distribution, metabolism and excretion. Any of these component variabilities might exhibit a polymorphism which could be completely hidden in the composite parameter of a single serum salicylate concentration value taken a fixed time after an oral dose.

Cummings & Martin (1962) were among the first to determine plasma and urine salicylate concurrently following dosage with aspirin. They pointed out that there was a time-interval between maximal plasma salicylate concentration and maximal rate of urinary excretion. Since the greater proportion of salicylate excreted in the urine is in the form of metabolites, these authors concluded that in the first few hours after the oral administration of a single dose of salicylate the relationship between the plasma concentration and the rate of removal of salicylate from the plasma is not always a simple proportionate one and that some other factors exercise an effect.

Nelson & Levy (1963) pointed out that when the urine was assayed by techniques permitting independent measurement of unchanged and meta-

bolized drug it was clear that the delayed excretion was due to salicyluric acid (a glycine conjugate of salicylic acid). Salicylic acid itself was excreted at a greater rate the higher the urinary pH. When the pH of the urine was elevated over 7.5 the free salicylate excretion could be accurately studied and occurred maximally about one hour after drug ingestion, thus coinciding with maximal plasma salicylate concentration. Salicyluric acid excretion is the terminal step of a two-step process—namely biosynthesis followed by excretion. Excretion is affected by urinary pH—occurring at a higher rate the more alkaline the urine, whereas the rate of biosynthesis is independent of the pH of the urine.

Salicylate elimination rates as assessed by decline in plasma salicylate concentrations were shown by Levy & Hollister (1964) to be fairly consistent in the same individual but to vary considerably between subjects. The suggestion is made by Levy (1965) that inter-subject variability may be due to a genetic polymorphism controlling maximum salicyluric acid formation capacity, but this idea awaits more extensive experimental verification.

#### **FAMILIAL INCIDENCE OF CHLORAMPHENICOL-PRODUCED APLASTIC ANÆMIA**

The evidence concerning chloramphenicol produced aplastic anæmia is fully reviewed by Meyler (1966). The incidence of this condition is stated to be between 1 of 500 and 1 of 100,000 persons exposed. Milder grades of marrow depression can be demonstrated in all subjects with plasma chloramphenicol levels of 25 µg/ml or more, but most people do not progress to the development of aplastic anæmia.

The possibility that there may be genetic predisposition to develop severe marrow damage is suggested by the report of Rosenthal & Blackman (1965). They report a pancytopenia in a 36-year-old, otherwise healthy, man treated for mild conjunctivitis on most days of 23 months with 0.5 per cent aqueous chloramphenicol eyedrops. Two years before this patient was seen his five-year-old niece—a daughter of the patient's sister—had died of an aplastic anæmia following the ingestion of chloramphenicol.

#### **GENETIC FACTORS IN HYPERSENSITIVITY (ALLERGIC) RESPONSES**

A high proportion of the adverse reactions to drugs are characterized by hypersensitivity phenomena, almost no drug being exempt, but certain drugs are notorious, e.g. penicillin. The clinical manifestations are variable and include not only urticaria and anaphylactoid œdema, but also purpura with and without thrombocytopenia, asthmatic attacks and circulatory collapse. In many instances the exact mechanism by which these reactions occur is obscure. However, in some instances it has been possible to correlate the patient's clinical state with the demonstration of a circulating antibody.

Ackroyd (1954) as a result of extensive studies on patients suffering from allylisopropylacetylurea (Sedormid)-induced thrombocytopenia found the following:

- (1) The addition of the responsible drug to the patient's platelet rich plasma gave agglutination, and if complement was present, lysis of platelets.

- (2) *Agglutination and lysis of normal platelets were obtained if both patient's serum and responsible drug were added to normal platelet rich plasma. These antibody-mediated effects produced inhibition of clot retraction.*

The sera of 16 patients with characteristic bleeding manifestations occurring after the taking of quinidine or quinine and subsiding when the drug was discontinued, have been investigated by Van Der Weerd (1965) by the platelet-agglutination and the complement fixation test. The serum of nine patients with quinidine-induced thrombocytopenia and of three patients with quinine-induced thrombocytopenia gave positive results in the complement-fixation reaction if small amounts of the responsible drug as well as platelets and complement were present. This antibody was not demonstrable in patients who had received the drugs without developing thrombocytopenia. The dextro- and levorotatory stereoisomers quinidine and quinine had different specificities; antibodies developed to the one do not give a positive result when tested against the other compound.

Jansz (1965) points out that the detection of antibodies against drugs is often difficult because the result of serological investigation depends on several factors. The drug often acts as a hapten—in other words it needs to combine with some larger molecule such as a plasma protein to become antigenic. The antigenicity of an ingested drug may depend on one of its metabolites. The antibodies to which a drug may give rise may have some properties making their detection with one method impossible while some other method is successful. An artificial binding of drugs onto the cell wall protein is obtained by treating erythrocytes and the drug with bis-diazotized benzidine. Incubation of drug-coated erythrocytes with patient's serum results in agglutination if antibodies are present. Using this technique the author and his collaborators have been able to demonstrate antibodies against acetylsalicylic acid, chloroquine, streptomycin, ACTH, amidopyrine, sulphonamide, sulphamerazine and PAS.

The problem arises as to why some individuals develop an antibody to a particular drug whereas others do not, and there is as yet no proper understanding of this difference. The possibility that an hereditary factor may well be responsible in part is raised by the painstaking work of Harris, Kalmus & West (1963) and West & Harris (1964). These workers found that about 25 per cent of Wistar rats from a particular colony failed to react to their first intraperitoneal injection of dextran (molecular weight about 140,000) and egg white. The usual reaction is an anaphylactoid one where the rat develops hyperæmia, pruritis and swelling of the snout and paws. If azovan blue dye is given intravenously at the same time as dextran, reactor rats show a blue colouration of the oedematous areas.

Genetic studies revealed that "non-reactivity" to egg-white and dextran is a recessive autosomal character whereas reactivity is dominant. The strains investigated other than Wistar did not show the polymorphism.

Extensive investigations failed to reveal the exact metabolic basis for the polymorphism, but it was shown that there was a ten-fold rise in the blood histamine levels of reactor rats, persisting for over two hours, after intravenous dextran. In non-reactor rats blood histamine levels remained un-

changed after dextran administration. Skin histamine and 5-hydroxytryptamine contents were higher in non-reactors than in reactors, indicating that their failure to respond was in no way due to a deficiency of the amines in the skin.

Dextrans of molecular weight over 100,000 are able to induce antibody formation in Man (Kabat & Bezer, 1958), but this requires an immunizing dose of the substance. Occasionally patients who have not previously been exposed to dextran develop an anaphylactoid reaction in response to a small dose of the chemical (see: Moorhead & Patel, 1964—Subject No. 10—for a typical example). It is possible that this type of person is a rare “reactor” phenotype in human populations of whom the majority of persons are of the “non-reactor” phenotype.

An unusual hæmolytic anæmia pointing to a new immunological mechanism of drug toxicity has been reported in response to methyl-dopa. Carstairs and co-workers (1966) had their attention alerted by finding three patients who had been on methyl-dopa with a positive direct anti-globulin (Coombs') test of a  $\gamma$  (warm) type. Then 104 patients receiving hypotensive therapy were investigated; and of 57 on methyl-dopa five had a positive direct antiglobulin test of a  $\gamma$  type, whereas amongst 47 on other forms of therapy none had a positive test. Generally drug-induced hæmolytic anæmia is caused by the drug acting as a hapten causing a non- $\gamma$  type of direct antiglobulin reaction. Further patients of this type are described by Hamilton, Jenkins & Turnbull (1966) and Paton (1966). A similar instance involving another drug is recorded by Cannat & Seligmann (1966) who draw attention to an abnormally high incidence of antinuclear factor in tuberculous patients treated with isoniazid. Here again there may be an interplay between a drug and genetic constitution to produce an immunological response—even though the patients seemed to be free of symptoms related to the antibody produced.

#### OPEN ANGLE GLAUCOMA—A PHARMACOGENETIC APPROACH

The disorder of open angle glaucoma is one of the major causes of blindness in temperate countries. The ætiology of the disorder is unknown and as the name implies there is no gross abnormality to be seen in the filtration angle of the eye. It is considered likely that some process takes place with increasing age in the trabecular mesh-work through which the aqueous humour filters before discharging into the canal of Schlemm. Although the net formation of aqueous humor is generally depressed in glaucoma, the difficulty in outflow causes an increased pressure inside the eye. This increased intraocular pressure damages the optic nerve, probably by interfering with its blood supply. It seems likely that the capacity of eyes to withstand raised intraocular pressure varies a great deal—so that very different degrees of visual disability result from the same rise of intraocular pressure.

Following the introduction of corticosteroid eye drops it became apparent that these drops when instilled for some days into the conjunctiva caused a great rise of pressure in an eye prone to open angle glaucoma, and that vision might be impaired. The pressure in the contralateral eye remained unaffected.

The changes in pressure and outflow facility which are produced in

normal and in glaucomatous eyes were carefully recorded by Armaly (1963) and by Becker & Mills (1963). The latter workers also observed the frequency distribution curves and found that both intraocular pressure and outflow facility had a suggestively bimodal distribution in 30 normal volunteers who were free of family history of glaucoma. 0.1 per cent betamethasone alcohol was instilled into one conjunctival sac in each of the volunteers four times daily for up to four weeks. There was a clear indication of the relationship between the age of the subjects and rise of intraocular pressure, and subjects over the age of 40 years attained intraocular pressure rises to 21 mm Hg (or more) four times more frequently than in subjects under 40 years. (There was a similar age distribution of responses to an oral water provocation test.) These changes were fully reversible on withdrawal of the steroid eye drops. Other corticosteroids (prednisolone, dexamethasone, hydrocortisone and triamcinolone) produce the same effects as betamethasone; but desoxycorticosterone acetate (DOCA) did not produce these responses.

Almost all the offspring of glaucomatous subjects, including those aged less than 40 years, gave the above responses (rise of pressure to 21 mm Hg or more and a positive water provocation test) when they were given conjunctival betamethasone drops (Becker & Hahn, 1964). This suggested very strongly that the response to topical corticosteroids was genetically determined.

Armaly & Becker (1965) have extended their study and suggest that intraocular pressure is controlled by a pair of allelic genes called  $P^L$  and  $P^H$  which determine respectively low and high intraocular pressure in response to steroid administration. Three combinations (or genotypes) are thus postulated  $P^L P^L$  and  $P^H P^H$  which are homozygotes and  $P^L P^H$  which is the heterozygote. The importance of these studies can be gauged from the fact that if the liability of  $P^L P^L$  to develop open angle glaucoma is arbitrarily assumed to be unity, then the liability of  $P^L P^H$  will be 15 and that of  $P^H P^H$  110. Thus the risk or susceptibility factor has high predictive significance.

These interesting studies, if confirmed, indicate a genetically determined variable which contributes to the liability to develop open angle glaucoma in later life. The suggested mode of action of the steroids is to produce a change in the mucopolysaccharide in the trabecular tissue in the filtration angle (Perkins, 1965).

### ANTICOAGULANT RESISTANCE

A very interesting family showing hereditary transmission of exceptional resistance to anticoagulant drugs has been described by O'Reilly and co-workers (1964). This family was located through observing as a propositus an otherwise healthy 73-year-old man who had sustained a myocardial infarct.

It was found that warfarin in a dosage of 20 mg daily had no significant effect on the prothrombin time, although warfarin absorption and the fall-off of plasma warfarin concentrations were shown to be within the range of normality previously determined by the same workers (O'Reilly, Aggeler & Leong, 1963). Thereafter the propositus was tested with a series of orally and intravenously administered doses of warfarin varying from 40 to 1,010 mg. Absorption was complete. plasma warfarin levels were proportional to dosage

and the recovery of a warfarin metabolite from the urine was similar to that found in normal subjects given equivalent amounts. Plasma protein binding of warfarin was the normal 97 per cent. In other words, all the evidence pointed to normal warfarin metabolism, and there was nothing to indicate any disease other than the myocardial infarct.

The prothrombin response of this patient, despite being proportional to the dosage of warfarin, was very much less at all dose levels than that of a normal person. The dose response curves shown in Fig. 7.6 have the same slope in both the propositus and a normal person, but in the former a much larger

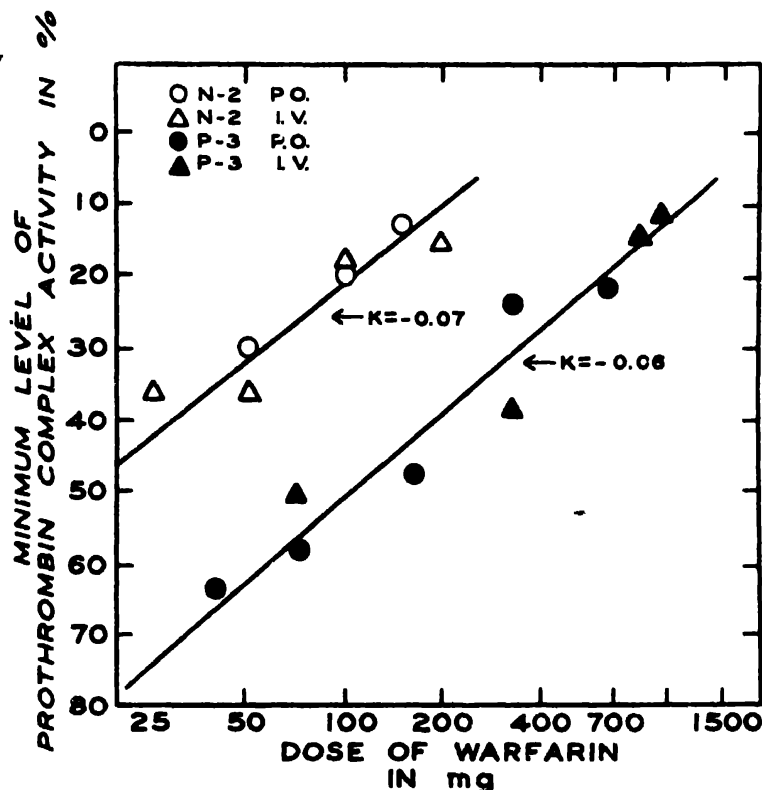


FIG. 7.6. Comparison of the Dose-Response relation in the propositus (P-3) and a normal subject (N-2).

The response is expressed on a linear scale as the minimum level of prothrombin activity achieved after the dose was administered and is plotted as a function of the log of the dose. Regression lines were fitted to the data by the method of least squares; the slope of each line is indicated by  $K$  (from O'Reilly *et al.*, 1964).

dose is needed to achieve a given response. The atypically small lowering of prothrombin activity was also observed in the propositus in response to bishydroxycoumarin and phenylindanedione. The effect of vitamin  $K_1$  (phytomenadione) on the hypoprothrombinæmic response to warfarin was much greater in the propositus than in a normal subject even though the doses of warfarin required to produce the hypoprothrombinæmia were much greater.

The wife of the propositus and seven relatives in three generations were tested with warfarin. Six of the relatives displayed the character of enhanced resistance to warfarin. No member of the family was under treatment with



any other drug during the course of these studies. Particular attention was paid to the avoidance of vitamin K and also barbiturates, since the latter are known to reduce the plasma concentrations and hypoprothrombinæmic effects of orally administered anticoagulants (Dayton *et al.*, 1961). The family data are consistent with the view that the atypical resistance to the drug is controlled by a single autosomal dominant gene or by an X-linked dominant one.

Information is totally lacking concerning the frequency in populations of persons with this type of inherited resistance to anticoagulants.

Olson (1964) speculates on clotting factor production as an "operon" (see below). Factors, II, VII, IX and X are possibly produced under the control of a series of structural genes actuated by an operator gene. A regulator gene produces a repressor substance whose repressive action is blocked by vitamin K. Anticoagulant drugs interfere with this repressor blockade effect of vitamin K. O'Reilly & Aggeler (1965) suggest that a possible basis for the altered response in their kindred is an atypical regulator gene producing an atypical repressor substance with a decreased affinity for anticoagulant drugs or an increased affinity for vitamin K (or both).

## THE RESPONSE TO SUXAMETHONIUM

### Idiosyncratic Sensitivity

Soon after the introduction of the relaxant suxamethonium into anæsthetic practice, occasional individuals were found who responded in an atypical manner. The customary short period of muscular relaxation was continued in these subjects giving rise to prolonged apnœa (Bourne, Collier & Somers, 1952; Evans *et al.*, 1952). The drug was known to be hydrolysed by the enzyme pseudocholinesterase, and measurements of the activity of this enzyme in the plasma disclosed low levels in these unusually sensitive subjects. Low levels of plasma activity are seen in liver disease, poisoning by organophosphorus compounds, carcinoma and after anticholinesterase drugs, but the unusually sensitive subjects did not have these causes.

A familial aggregation of low plasma pseudocholinesterase activity levels was described by Lehmann, Silk & Liddell (1961), although the technique used allowed neither phenotypes nor genotypes to be differentiated from each other with clarity.

An improved technique which allowed the actual definition of genotypes was introduced by Kalow and Genest (1957). This involved the estimation of the percentage inhibition by the local anæsthetic dibucaine (cinchocaine) of the action of plasma pseudocholinesterase on benzoylcholine. The result is expressed as the dibucaine number (DN). Using this technique the suxamethonium-sensitive subjects were shown to be homozygous, and their parents heterozygous, for a gene determining low pseudocholinesterase levels. Various other biochemical tests indicated that allelic genes controlled alternative pseudocholinesterase structures (Davies, Marton & Kalow, 1960).

A further variant of the enzyme was defined by Harris & Whittaker (1961). They found that pseudocholinesterase was inhibited by fluoride and that this inhibitor, like dibucaine, differentiated genotypes. The genotypes defined by the two inhibitors were not identical, and further family studies indicated

TABLE 7.1  
HEREDITARY VARIANTS OF PSEUDOCHOLINESTERASE RESULTING FROM FOUR ALLELIC GENES  
(after: Kalow, 1965a)

Genotype	Phenotype	Prevalence	Enzyme Status	Response to Succinylcholine	DN	FN
<i>Homozygotes</i>						
$E_1^u E_1^u$	U	1 : 1	Usual type of esterase	Normal	80	60
$E_1^u E_1^a$	A	1 : 2,500	Atypical esterase	Grossly prolonged	20	20
$E_1^u E_1^f$	S	1 : 100,000	No activity	Grossly prolonged		
$E_1^f E_1^f$	F	rare	Insufficiently investigated	Prolonged	70	30
<i>Heterozygotes</i>						
$E_1^u E_1^a$	I	1 : 25	Mixture of enzymes	Almost normal	60	45
$E_1^u E_1^f$	UF		Mixture of enzymes	Almost normal	75	50
$E_1^a E_1^f$	IF		Mixture of enzymes	Prolonged	45	35
$E_1^u E_1^s$	U	1 : 200	Usual type, decreased activity	Almost normal	80	60
$E_1^a E_1^s$	A	1 : 80,000	Atypical esterase, decreased activity	Grossly prolonged	20	20
$E_1^f E_1^s$	F		Not observed	(Prolonged)	(70)	(30)

## KEY TO SYMBOLS

$E^u$ ,  $E^a$ ,  $E^f$  and  $E^s$  = Alleles controlling the usual, dibucaine-detected atypical, fluoride-detected atypical, and silent, forms of pseudocholinesterase respectively.

Phenotypes: U = Usual  
 A = Atypical  
 F = Fluoride  
 S = Silent  
 I = Intermediate

} forms of pseudocholinesterase as determined by DN and FN.

DN = Dibucaine number = percentage inhibition of enzyme activity produced by dibucaine;  
 FN = Fluoride number = percentage inhibition of enzyme activity produced by fluoride.

Information in brackets predicted not observed.

that there was yet a third allele controlling the structure of the enzyme plasma pseudocholinesterase (Harris & Whittaker, 1962).

Liddell, Lehmann & Silk (1962) described a Greek woman who seemed to possess no plasma pseudocholinesterase, but whose offspring had plasma enzyme activity values a little below normal, behaving normally towards inhibitors. This family was evidence in favour of a fourth "silent" allele. Further examples of families yielding evidence for the "silent" gene have been published by Szeinberg, Pipano & Ostfeld (1965), Dietz, Lubrano & Rubinstein (1965) and Simpson & Kalow (1964). Hodgkin and co-workers (1963) could find no evidence for any pseudocholinesterase-like protein in a subject presumed homozygous for the "silent" allele but Goedde, Gehring & Hofmann (1965) were able to show in a similar case two to three per cent of the usual activity. Furthermore a specific antiserum was absorbed by the "silent gene" serum as it was by serum containing the usual form of the enzyme, although certain differences existed in immunological behaviour between usual and "silent gene" enzyme proteins. It seems likely that this allele like the other three produces structural changes in the enzyme molecule.

The frequencies of the various genotypes due to this system are shown in Table 7.I.

A reliable screening test for sera containing the atypical enzyme has been devised by Harris & Robson (1963). This simply performed agar-diffusion test uses "RO2-0683 Roche" as the discriminating inhibitor. About 200 sera can be screened overnight and "atypical" sera which show little inhibition can then be further investigated by the more time-consuming spectrophotometric methods (Simpson & Kalow, 1965).

#### **Idiosyncratic Resistance**

A very interesting family which is in a sense complementary to the above observations, has been described by Neitlich (1966). In a survey of sera from 1,029 military personnel, one was found to have a plasma pseudocholinesterase activity of 1,278 units, which was about three times as great as the mean value for the remainder ( $391 \pm 0.75$ ). This man had a much smaller impairment of grip following a small dose of suxamethonium than was observed in controls.

Serum from the mother, sister and daughter of the propositus all showed the characteristics described above. The evidence suggests therefore that in this family an autosomal dominant gene controls the structure of an enzyme molecule which is much more active than the usual pseudocholinesterase.

In order to decide whether this gene is at the same locus as those conferring increased susceptibility to suxamethonium rare families showing segregation (or non-segregation) of two alleles need to be studied.

#### **Electrophoretic Polymorphism without influence on Drug Sensitivity**

Harris, Hopkinson & Robson (1962) performed two-way electrophoresis of serum first on paper and secondly on starch.

An additional slow-moving zone of pseudocholinesterase termed  $C_2$  was consistently found in 14 individuals out of 300 randomly selected adult individuals. Sera possessing this zone had about 30 per cent more enzyme

activity than controls of the usual type (Harris, Hopkinson, Robson & Whittaker, 1963). It seems that the genes controlling this polymorphism are at a locus different from that controlling the suxamethonium-sensitive genotypes referred to above, which was designated E, as indicated in Table 1.

### **A General View of Complex Polymorphisms**

The many varieties of pseudocholinesterase which can occur in the population through the operation of genetic mechanisms should not be regarded as in any way exceptional. The more intensively any given protein or enzyme is studied, the more varieties are found. This view is substantiated by studies on hæmoglobin variants (Huehns & Shooter, 1965) alkaline phosphatase and other enzymes (Harris, 1966). It is clear that many alternative alleles can occupy a location on a chromosome and as a result many different types of protein product can be found in the population. This can be of practical importance in medicine away from the purely biochemical field, e.g. in skin transplantation studies in the mouse 18 alleles have been demonstrated at one locus only (locus "H2") and these alleles exert a significant influence on the fate of grafts (Snell *et al.*, 1964). Recent work (Dausset, Ivanyi & Ivanyi, 1965) suggests that similar genetic mechanisms operate in human organ transplantation problems.

### **ACATALASIA**

This condition was discovered as a result of a clinical observation made by Takahara in 1946 (Takahara, 1952). When hydrogen peroxide was applied to a bleeding wound in a patient with oro-nasal sepsis, instead of the usual bubbles of oxygen a brownish-black colour appeared. Investigation of this patient showed that she did not have catalase in her red cells. Subsequently, other similar patients have been found and the enzyme has been shown to be absent not only from red cells but also from the tissues of the nasal and oral cavities, the liver and the bone marrow (Takahara, 1961). About half Asiatic acatalasics have oral sepsis as in the first case observed.

Using a precise enzymological assay technique it has been shown that acatalasics are homozygous, and their parents heterozygotes (Nishimura *et al.*, 1959). Electrophoretic and immunological studies reveal a complete lack of catalase-like protein in Asiatic acatalasics.

Aebi and co-workers (1962) screened blood samples from 18,459 persons in Switzerland and found two acatalasics. The red cells in these subjects possess about one per cent of the catalase activity found in normal red cells. European acatalasics do not appear to suffer oro-nasal sepsis of the type found in Asiatic acatalasics.

### **PTC TASTE-TESTING POLYMORPHISM**

Fox (1932) prepared phenylthiocarbamide (synonyms: phenylthiourea; PTC) and found that some persons detected a bitter taste easily when the crystals were placed on the tongue—and these were found to possess the gene controlling the dominant character. Other persons could only detect a slight taste and these were recessives constituting about 33 per cent of European and a much lower percentage of African and Chinese populations. Harris & Kalmus (1950) introduced a more precise way of defining these

phenotypes and by testing a panel of individuals with various chemical compounds it became possible to determine the chemical linkage required to detect the polymorphism (Barnicot, Harris & Kalmus, 1951). This linkage  $S = C \begin{smallmatrix} \diagup \\ \diagdown \end{smallmatrix} NH^-$  is present in thiouracil (and derivatives) and thiopentone, solutions of which can be employed to differentiate "tasters" from "non-tasters".

The following associations have been shown to exist between the phenylthiocarbamide-tasting phenotype and thyroid disease:

- (1) a higher incidence of non-tasters among subjects with adenomatous goitre (Kitchin *et al.*, 1959; Harris, Kalmus & Trotter, 1949);
- (2) a higher incidence of tasters among subjects with toxic diffuse goitre (Kitchin *et al.*, 1959);
- (3) a higher incidence of non-tasters among subjects with athyreotic cretinism (Fraser, 1961).

Another disorder found to be associated with the PTC taste testing polymorphism is primary open angle glaucoma. Becker & Morton (1964) assessed almost 2,000 patients with the testing technique of Harris & Kalmus (1950) except that they did not employ the eight glass procedure advocated by these latter authors. Becker & Morton (1964) found a high incidence of non-tasters, of both Caucasian and Negro ethnic groups, in open angle glaucoma patients who had definite field loss. By contrast angle closure glaucoma subjects (again in both ethnic groups) were deficient in non-tasters of PTC, and this is known to be a quite different type of ophthalmic disorder.

As a result of their studies McLenachan & Davies (1965) and Becker, Kolker & Ballin (1966) have further investigated the relationship between thyroid function and glaucoma. The latter team found serum protein bound iodine values to be much lower in open angle glaucoma than in angle closure glaucoma. Also the 24 hour radio iodide uptake was low in open angle as compared with angle closure glaucoma.

The metabolic basis of associations between PTC tasting phenotypes and both thyroid disorders and glaucoma remains obscure.

Evans, Kitchin and Riding (1962) failed to demonstrate any difference in the metabolism of methyl-thiouracil and thiopentone between tasters and non-tasters, and argue that the metabolic basis of the polymorphism may well be localized to particular tissues.

## SULPHONAMIDES AND HÆMOGLOBINOPATHIES

### Hæmoglobin Zürich

This hæmoglobin mutant in a European family was discovered as a direct result of treatment with a sulphonamide drug. A father and his small daughter both suffered severe hæmolytic anæmia following such treatment and it was observed that the red cells contained inclusion bodies (Hitzig *et al.*, 1960; Hitzig, 1961).

Electrophoretic studies showed that these subjects possess a mixture of two hæmoglobins A and Zürich—which has a mobility between A and sickle hæmoglobin. Analysis of peptic digest "fingerprints" reveal three unusual

peptides whose presence may be explained by the replacement by an arginine residue of the histidine residue normally present in position 63 of the  $\beta$  chain of hæmoglobin.

The members of the family have either hæmoglobin A alone or a mixture of A and Zürich. Ferrokinetic and  $^{51}\text{Cr}$ -tagged red cell survival studies showed that red blood cells from the original patients had a shorter life than normal in the circulation. It was also observed that some of the heterozygous persons suffered from anæmia and mild episodes of jaundice in the absence of drug therapy.

By means of an *in vitro* test using  $^{51}\text{Cr}$ -tagged cells, it was shown that the subjects' red blood cells are lysed with abnormal readiness in the presence of primaquine and Causyth (8-/dimethylaminoantipyrin/oxyquinoline sulphonic acid) in addition to a variety of sulphonamides. Other drugs, however, including acetylsalicylic acid and phenobarbitone, showed no such effect (Frick, Hitzig & Betke, 1962; Bachmann & Marti, 1962).

### Hæmoglobin H

Whereas hæmoglobin A consists of two  $\alpha$  chains and two  $\beta$  chains ( $\alpha_2\beta_2$ ), hæmoglobin H is a tetramer of four  $\beta$  chains ( $\beta_4$ ). Its presence gives rise to a hæmolytic anæmia which may be partly due to the abnormal properties of hæmoglobin H. For instance, the susceptibility, greatly increased by oxidation to methæmoglobin, of hæmoglobin H to denaturation and precipitation, leads to the formation of insoluble intraerythrocytic inclusions in red blood cells aged 40 to 45 days and to rapid destruction of these erythrocytes by the spleen. In addition, reduced hæmoglobin H is less soluble than reduced hæmoglobin A, and precipitation of erythrocytes in capillaries causes increased cell destruction.

Rigas & Koler (1961) noticed that in one of their subjects the administration of sulphisoxazole for urinary tract infection caused an abrupt drop in the erythrocyte survival curve measured with radioactive chromium. They treated red blood cells from subjects with hæmoglobin H *in vitro* with sulphisoxazole, amyl nitrite, sodium nitrite and methylene blue. Hæmolytates prepared thereafter showed hæmoglobin A only, the hæmoglobin H having been precipitated. Microscopy of red blood cells from subjects with hæmoglobin H incubated with these substances showed more numerous and larger intraerythrocytic inclusion bodies than did cells from normal subjects. These observations strongly suggest that hæmoglobin H *in vivo* is liable to denaturation in the presence of drugs.

## MISCELLANEOUS OBSERVATIONS

### Diabetes Mellitus and Insulin

A technique for estimating insulin activity was devised by Vallance-Owen, Hurlock & Please (1955) by measuring the uptake of glucose by a rat hemidiaphragm. An insulin antagonist was demonstrated to be associated with plasma albumin and to be present to a much higher concentration in diabetic subjects than in non-diabetics (Vallance-Owen, Dennes & Campbell, 1958). The inhibition of insulin activity on a standard rat-diaphragm preparation can be quantitatively assayed for plasma samples when their albumin con-

centration is reduced to 1.25 g per 100 ml. The frequency distribution histogram of this parameter is bimodal (Vallance-Owen, 1963). Persons with enhanced antagonism are referred to as "synalbumin positive" (approximately 20 per cent of the British population) and those in the other mode as "synalbumin negative". Ensink, Mahler & Vallance-Owen (1965) have demonstrated that the antagonist bound to the plasma albumin is the  $\beta$  chain of insulin. Family investigations show that the "synalbumin positive" state is a Mendelian dominant character, and it is suggested that for a person to develop idiopathic diabetes mellitus, he must be "synalbumin positive". Vallance-Owen & Ashton (1963) have further shown that persons with the character "synalbumin positive" seem more prone to develop myocardial infarction even though they are not in fact diabetic by the usual clinical and biochemical criteria.

These studies have been substantially confirmed by various workers, e.g. Alp & Recant (1964) and Kammerer *et al.* (1966), although not by Keen (1963). They constitute a valuable step forward in our understanding of the genetic factors predisposing to the development of both diabetes mellitus and myocardial infarction.

#### Phenothiazines

The role of heredity in drug-induced Parkinsonism has been studied by Myrianthopoulos, Kurland & Kurland (1962). A survey was made of 728 relatives of 59 probands who had symptoms of Parkinsonism when receiving phenothiazine drugs and of 777 relatives of 67 control subjects who proved resistant to the Parkinsonian effects of the same drugs. Among the relatives of the probands, 13 were found to have Parkinson's disease and among the relatives of the control subjects three were found to have Parkinson's disease. These numbers are too small to be statistically significant, but they do suggest that further examination of the extrapyramidal side-effects of phenothiazines might be worth undertaking.

As far as the author is aware, there are no genetic observations on chlorpromazine jaundice.

#### Antidepressants

Pare, Rees & Sainsbury (1962) investigated the response of depressed patients to amine oxidase inhibitors, and the imipramine group of antidepressives. They were interested in whether patients responded similarly in succeeding illnesses and whether the response to therapy with drugs was comparable in first-degree relatives who also became depressed. Consistent responses in the same patient were more than twice as frequent as inconsistent responses, and in 12 intrafamilial comparisons the results were similar in proband and relative. In five of these 12 intrafamilial comparisons improvement occurred in both patients, whilst in the remaining seven comparisons neither the patient nor the relative had benefited from treatment with the drug. It is suggested by these authors that the response to monoamine oxidase inhibitors, on the one hand, and the imipramine group of antidepressants on the other, differentiates two genetically specific types of depression. This inference drawn by Pare and co-workers (1962) is challenged by Crisp (1963), who regards the data as insufficient to be conclusive. However, this approach

to mental disease must prove stimulating as it is well known that similar types of psychotic disorders tend to run in families (Hurst, 1961).

### **The Operon and Induction Phenomena**

This sub-section is included, although there are no fully proven examples of polymorphisms governed by operons in Man, because of the exciting possibilities that pharmacogenetic phenomena may be found in this area in the future.

As a result of studies on the induction of enzyme synthesis in bacteria, Jacob & Monod (1961) discovered new categories of genes called "regulators" and "operators" which influence the rate of production of proteins whose molecular configuration is determined by "structural" genes. An operon is that segment of genetic material (desoxyribonucleic acid = DNA) which bears a functionally coordinated multigene complex of operator and structural gene(s). Figure 7.7 shows a general model for this concept which has been largely proven by experiments in micro-organisms.

Kalow (1965b) presents the situation thus:

"The biological importance of the regulator genes should not be underestimated; it seems that many structural genes are repressed most of the time. For illustration, let us recall that all cells of any given organism contain the same genes. The difference between cells, e.g. between a nerve and a muscle cell, indicates that different sets of genes are in operation. The inactive genes are presumably repressed. Enzyme induction is a de-repression of genes, whereby the enzyme inducer is thought to combine with the repressor causing its inactivation."

It has been known for some time that patients receiving barbiturates are resistant to anticoagulant therapy and require larger doses than unpremedicated patients in order to produce a given prothrombin response (Dayton *et al.*, 1961). This phenomenon has also been studied by Cucinell and co-workers (1965) who demonstrated that phenobarbital was effective in Man in lowering plasma bishydroxycoumarin and diphenylhydantoin concentrations. Similarly Chen and co-workers (1962) were able to show greatly accelerated demethylation of intravenously administered aminopyrine to 5-aminoantipyrine in persons who had received oral medication with phenylbutazone for ten days. Giving the phenylbutazone intravenously immediately prior to the aminopyrine infusion did not, however, influence the metabolism of the latter drug.

The explanation for these phenomena of "enzyme induction" comes from studies of a similar nature in laboratory animals. When phenobarbitone is given to guinea-pigs, rats, rabbits or dogs, the liver cells can be shown by electron microscopy to develop increased amounts of endoplasmic reticulum. In addition, these cells can be demonstrated to synthesize increased amounts of microsomal enzyme protein (Burns, Conney & Koster, 1963). These changes are preceded by a temporary increase of microsomal cytochrome concentration (Remmer & Merker, 1965).

It thus appears that the chronic administration of various hypnotics, tranquillizers, antihistaminics, antirheumatics, analgesics and muscle relaxants greatly increases the amount of drug-metabolizing enzymes in



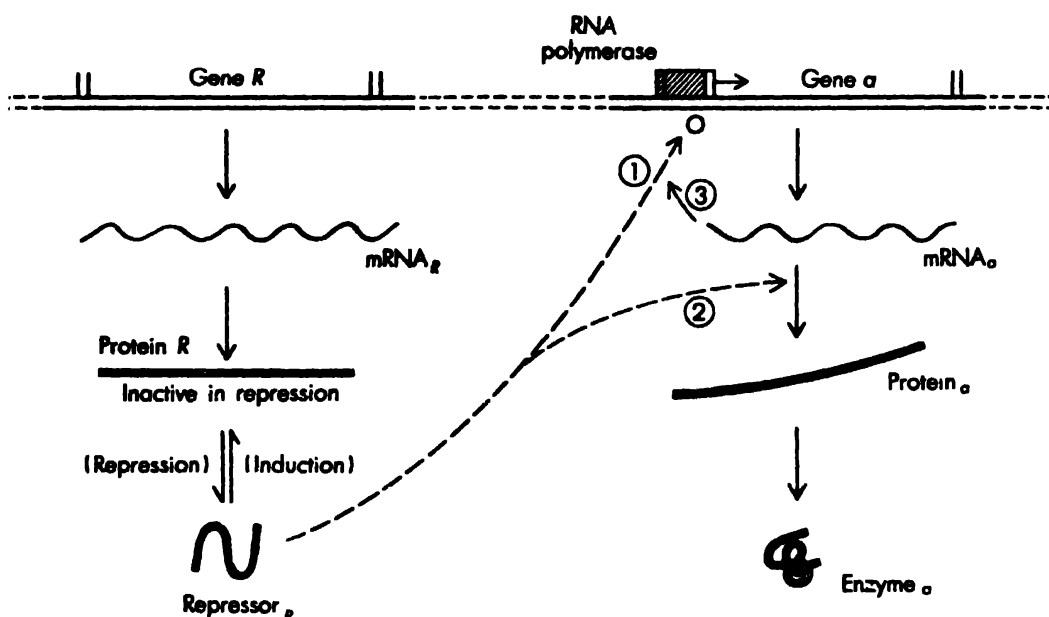


FIG. 7.7. A general model for induction and repression of specific protein biosynthesis. The figure shows some of the major components that are presumed to take part in the regulation of genic activity and some possible sites of interaction by the components.

A gene, *R* (regulator), is responsible for the synthesis of a protein that can exist in two conformations. In one conformation the protein is inactive as a repressor. In the second configuration (bottom) the protein has affinity for a chromosomal location, *O* (operator) (step 1). When the repressor protein is at *O*, RNA polymerase is unable to synthesize messenger RNA along structural gene  $\alpha$ . When repressor protein is absent from *O*, RNA polymerase initiates synthesis of messenger RNA. A modification of this general scheme is diagrammed as reactions 2 and 3. In this modified model, the repressor acts at the level of messenger RNA, causing it to remain complexed with the structural gene and to halt further messenger RNA synthesis at this place on the chromosome.

The amount of the repressor available for inhibition of messenger RNA synthesis depends upon the rate of synthesis of the repressor protein, upon the repressor's affinity for *O*, and upon the presence of molecules of small molecular weight that influence the interconversion of the two forms of the repressor. In induction, the metabolite maintains the repressor molecule in a form that is inactive as a repressor. In repression, the metabolite enhances the conversion of repressor molecules from the inactive form to a conformation that is active as repressor (after Hartman & Suskind, 1965).

liver microsomes. These enzymes include those subserving N-dealkylation, ether cleavage, hydroxylation, deamination, and glucuronide formation.

Enzyme induction affects not only the metabolism of drugs, but also natural substrates. Conney and co-workers (1965) have shown that treatment of guinea-pigs with diphenylhydantoin or phenobarbital for several days gives rise to a marked increase in the activity of an enzyme system in liver microsomes responsible for the 6- $\beta$ -hydroxylation of cortisol. This finding probably explains the increased excretion of 6-hydroxycortisol which has been found in humans under treatment with phenobarbital or diphenylhydantoin, since it is known that the increased 6-hydroxycortisol excretion arises from increased extra-adrenal hydroxylation of cortisol.

Granick (1965) has shown that barbiturates, and other drugs which precipitate attacks of acute intermittent porphyria in Man, can induce

synthesis of hepatic  $\delta$ -amino levulinic acid-synthetase when they are given to animals or added to cell-culture preparations. He suggests that there may be a polymorphism in the operon controlling the manufacture of this enzyme in Man. According to this theory some individuals have a much less active repressor of  $\delta$ -ALA-synthetase formation than the general run of mankind. Under the influence of certain drugs these susceptible subjects are thought to manufacture far too much of this enzyme, giving rise to the formation of excessive porphyrins and the characteristic clinical picture. Tschudy (1967) has produced an important item of information supporting the validity of Granick's concept. A patient who died in an exacerbation of acute intermittent porphyria was shown to have a much higher level of  $\delta$ -ALA synthetase activity in the liver than was present in control specimens of liver obtained from normal people at laparotomy and autopsy. The levels of other enzymes including catalase were normal in this porphyria patient's liver.

Hormones are capable of acting as inducers and suppressors. A striking example is that of insulin. Weber & Singhal (1965) have demonstrated that insulin is an inducer of the key glycolytic and glycogenetic enzymes glucokinase, phospho-fructokinase, pyruvate kinase and glycogen synthetase. In addition this hormone suppresses the complementary gluconeogenic enzymes. Genetic polymorphism could of course exist in this insulin-influenced operon, and hence there is clearly a need to re-examine certain aspects of diabetes mellitus.

Another connected area of pharmacogenetics is opened up by the finding that antibiotic drugs may interfere with protein synthesis in various ways. Actinomycin forms a tight irreversible complex with DNA so that the formation of ribonucleic acid (RNA) is blocked (Reich, 1963). Puromycin interrupts the formation of peptide chains. Streptomycin alters the ribosome of the cells so that the messenger RNA is "mis-read" which results in a change in the structure of proteins being synthesized. This may be the way in which streptomycin acts as an antimicrobial agent (Gorini, 1966). These drugs therefore are powerful new tools in the study of the genetic control of the production-rate and structure of proteins.

Possibly of great importance in the field of nutritional medicine is the discovery that not only drugs and other exogenous compounds, but also differing types of diet, e.g. high carbohydrate or high protein, are capable to inducing substantial changes in the quantitative pattern of liver enzymes (Nutrition Reviews, 1965).

The studies which have been referred to in this section demonstrate the importance of environmental influences upon intracellular events. The mechanisms by which they bring about their effects may well be governed by genetic polymorphisms and provide models which allow the interaction of environmental and genetic phenomena to be explored at a biochemical level.

### **Pharmacogenetics in Experimental Animals**

Since animal work is often complementary to human studies, examples have already been referred to in this review. This subject is fully dealt with in a recent monograph (Meier, 1963), and it is evident that close parallels to some of the polymorphic systems described above in Man exist in animals:



was unimodal. There was no demonstrable relation between age or sex of the subjects and their drug half-life. Attempts to fit the family data to a single-gene mechanism failed. In an attempt to assess the possible role of multiple genetic factors operative in this system correlation coefficients between relatives were computed. The sib-sib correlation of corrected dicoumarol  $t\frac{1}{2}$  was  $0.347 \pm 0.091$ . No parent-child or midparent-midoffspring correlations could be demonstrated. Since non-genetic causes, especially "maternal effects", are likely to make a sizeable contribution to sib-sib correlations, in the absence of a midparent-midoffspring correlation it cannot be proven that the sib-sib correlation definitely indicates a hereditary component.

A second method which can be employed is to study people having known phenotypic characters and see whether these characters contribute to the variance of a continuous unimodal frequency distribution curve. This procedure has been adopted by Horwich & Evans (1966), who studied the influence of the three factors sex, A and O blood group, and salivary ABH secretor status on the cell-removing effect of aspirin on human gastric mucosa. The numbers of cells shed were estimated by determining the DNA content of gastric washings by the technique of Croft (1963). The results for the 160 persons studied in the experiment were normally distributed. A factorial analysis showed that sex made a significant contribution to the variance in that males shed more cells than females. Blood group and secretion did not have any significant effects when acting singly. There was a significant interaction between blood group and secretion; in persons of blood group O, secretors shed more cells than non-secretors; whereas in persons of group A the converse was true. There was a significant interaction between blood group and sex; in persons of blood group O, males shed more cells than females; whereas in persons of group A the converse was true.

The analysis of unimodal frequency distribution curves of drug metabolism or action in terms of genetic factors is a very new area, likely to yield considerable information of interest. For example, if the presence of a definite heritable component in the variance of a unimodal curve could be demonstrated by offspring-parent regression, then it would suggest that discontinuous phenotypic biochemical entities such as enzyme polymorphisms might profitably be sought.

## CONCLUSIONS

The finding of discontinuous variability of drug action or drug metabolism in human populations indicates the existence of polymorphic genetic systems, and the value of drugs as tools revealing the presence of new human genes. The most obvious clinical clues to the existence of a polymorphic system may be the occurrence in a few persons of striking side-effects (e.g. suxamethonium sensitivity), or a difference in reaction between racial groups (e.g. primaquine hæmolysis) as pointed out by Motulsky (1957).

The study of chronic diseases is aided by analysis of their genetic components. To pursue this approach, knowledge of more human polymorphic systems is required, and drug-detected systems are applicable to this end (e.g. steroids in glaucoma).

The factors perpetuating most of the polymorphisms described are

unknown. Malaria may play a part in maintaining some G6PD polymorphisms.

Polymorphisms of the order of isoniazid inactivation (50 per cent of each phenotype in Caucasian subjects) or glucose-6-phosphate dehydrogenase deficiency (up to 25 per cent in Negro subjects) are capable of interfering with the validity of double-blind trials, particularly when groups tested with a new drug are small. These polymorphisms introduce a variability into the groups which may lead to false conclusions unless the possibility of their existence has been eliminated by preliminary study.

With more new drugs of great therapeutic usefulness being produced each year, there is an increasing likelihood that genetic variability will be observed, not only in response to or metabolism of a single drug, but also with regard to combinations of drugs. Hence in the future, drug treatment may have to be planned on a much more personal basis for each patient than is now the case.

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## CHAPTER 8

# SYSTEMIC MYCOSES ✓

by

I. SARKANY

THE rise in incidence of the deep fungus infections is generally accepted and can be attributed to two factors. Firstly, there is a heightened awareness of this group of diseases, and secondly it is now recognized that certain modern treatments, particularly systemic steroids and broad spectrum antibiotics predispose to the development of systemic mycoses and that occasionally they may complicate various forms of malignant diseases. Whereas the teaching of infectious diseases due to bacteria, rickettsia and viruses is, generally speaking, part of the undergraduate and certainly of the postgraduate standard syllabus, there is still little systematic teaching about fungi. The dermatologist encounters and teaches a basic amount about the dermatophytes but the systemic mycoses do not receive much attention. Pulmonary aspergillosis, for instance, is not as rare a cause of chronic chest disease in England (Campbell & Clayton, 1964) as is generally supposed and reports of systemic candidiasis are becoming more frequent. The recognition of systemic mycoses may be rare by comparison with bacterial and viral diseases but their role in the production of disease must be appreciated, particularly because some of these, fatal when left untreated, can be controlled with recently developed anti-fungal antibiotics.

Emphasis is usually placed on the saprophytic behaviour of fungi or on their role as agents causing disease. Conant (1962), however, discusses their importance, not often stressed, as tools for biological research, particularly in genetics and chemistry, and quotes two fascinating examples of their use in this context. When the pharmacological effect of cortisone and hydrocortisone or their derivatives in rheumatoid arthritis was found to be dependent on the oxygen atom in the 11-position in the steroid nucleus, there were many attempts to make such compounds from steroidal substrates by chemical synthesis. Whereas many steps were necessary to introduce oxygenation at the 11-position by chemical methods, several fungi (*rhizopus*, *cunninghamella*, *aspergillus*, *streptomyces*, etc.) were found to be able to introduce by oxidation, the oxygen atom at this position by a one-step process. The second and more widely known role of fungi in the field of biological research is their use in the production of penicillin and innumerable other antibacterial antibiotics.

In this brief review it is proposed to discuss systemic fungus infections only. The systemic mycoses encountered in this country will be dealt with in more detail, i.e. candidiasis, aspergillosis, cryptococcosis and actinomycosis. Others, such as histoplasmosis, coccidioidomycosis and blastomycosis which are more restricted in their geographical distribution are dealt with

briefly since these diseases are only seen in patients who have travelled in endemic areas or handled materials sent from these areas. They may also occur in laboratory workers.

### Classification

/ Fungus infections are generally divided into the superficial and the deep forms. The superficial type is restricted to the skin, hair and nails, i.e. structures containing keratin. In the deep mycoses, deeper lying structures, including the dermis, the lungs, other viscera and bones, are involved. The systemic mycotic infections can be grouped as follows (Riddell, 1964):

1. Infections due to actinomycetes (filamentous organisms of bacterial dimensions).  
(a) Actinomycosis, (b) nocardiosis.
2. Infections due to yeasts and yeast-like fungi.  
(a) Candidiasis, (b) cryptococcosis (torulosis).
3. Infections due to filamentous fungi.  
(a) Aspergillosis, (b) mucormycosis.
4. Infections due to dimorphic fungi.  
(a) Coccidioidomycosis, (b) histoplasmosis, (c) North and South American blastomycosis, (d) sporotrichosis.

In addition a number of subcutaneous infections due to fungi exists. These include mycetoma, an indolent infection affecting the feet or hands. It may be caused by aerobic and anaerobic actinomycetes and by a number of other fungi. Similarly, chromoblastomycosis, a granulomatous infection of the skin seen in warm climates, may be caused by several pigmented fungal species.

### Epidemiology

The deep human mycoses are acquired essentially by inhalation or occasionally inoculation, i.e. sporotrichosis. However, *Actinomyces israelii*, the causative agent of actinomycosis, does not, as far as is known, exist as a saprophyte outside the human host. It is a well established component of the human mouth, and intimate oral contact, such as naturally occurs between mother and child, is probably one of the principal means of transference of this anaerobic actinomycete from one generation to another (Ajello, 1962). The organism lives in balance with its host and microbes in the body. Only under special conditions, such as are produced by mechanical trauma, does invasion of tissues and pathological change occur.

*Candida albicans* is normally a component of the cutaneous, oral, vaginal and intestinal flora of the human body. Predisposing factors to clinical disseminated candida infection in man are neoplastic diseases, reticuloses, chronic debilitating infections and the use of wide spectrum antibiotics and systemic corticosteroids. Under these conditions there is an alteration in the body's defence mechanisms, which allows the normally saprophytic *Candida albicans* to become pathogenic. Roth *et al.* (1959, 1961) showed that human blood normally had an anti-candida serum factor which was depressed in infants up to the age of six to eight months and was also deficient in patients

suffering from acute leukæmias, chronic terminal leukæmia, Hodgkin's disease, multiple myeloma and erythemic myelosis.

Histoplasmosis, coccidioidomycosis, cryptococcosis and aspergillosis are acquired by the inhalation of infective spores. These fungi occur in soil. There they presumably proliferate and produce reproductive spores and infectious mycelial elements which, under appropriate climatic conditions may be dispersed by air currents into the environment (Ajello, 1962). Some of the fungus cells may then be inhaled and give rise to infections. Only two species of fungi involved in airborne infections have so far not been isolated from soil: *Blastomyces dermatitidis* and *Paracoccidioides brasiliensis*. The suitable climatic conditions are probably responsible for the localization of these fungi in endemic regions, but transport of material contaminated with pathogenic fungus spores may spread the disease into non-endemic areas.

The ecological factors relating to the geographical distribution of *Coccidioides immitis* have been studied by Maddy (1957). Climate appears to be the main factor which defines the limits of the endemic regions of coccidioidomycosis in the United States. The climatic conditions of the Lower Sonoran Life Zone which encompasses the south-western parts of the U.S.A. apparently provide ideal biological support for *C. immitis* in the soil. High summer temperatures, mild winter, annual rainfall ranging from 5 to 20 inches, mainly during the winter, a certain altitude and characteristic flora (the creosote bush) are the requisites of this fungus. Finally, the quality of the soil is critical and appears to be more suitable in the immediate vicinity of burrows of various rodents at the base of shrubs. The higher humidity, lower temperature and rodent excreta of these parts of the soil are likely to contribute to the nutrient requirements of *C. immitis*.

Similarly, the endemic distribution of *Histoplasma capsulatum* and *Cryptococcus neoformans* depends on the factors which affect the quality of the soil where these fungi are found. *Histoplasma capsulatum* is especially prevalent in bird and bat habitats, in soil fertilized by chicken manure and in soil from around outbuildings and chicken coops. Infection has also resulted from soil contaminated by starlings in old derelict houses and from exposure to dust in caves inhabited by nocturnal bats in Venezuela and in South Africa where an influenza-like illness in cave-workers is not uncommon from exposure to *H. capsulatum*.

The critical differences in the nutritional requirements of the various species of fungi are very well illustrated by comparison between *H. capsulatum* and *C. neoformans*. Whereas the former thrives in chicken droppings, *C. neoformans* has been regularly found in pigeon droppings in various widely separated regions of the world, including Great Britain. Virulent strains of *C. neoformans* were first isolated from pigeon habitats in London by Randhawn and colleagues (1965). The association between virulent *C. neoformans* strains and pigeon excreta was later confirmed in London by others (Partridge & Winner, 1965).

Traumatic inoculation is the mode of transmission of a large group of deep mycotic infections which are encountered in warm climates but which are seen only exceptionally in England. These include sporotrichosis, chromoblastomycosis, mycetoma (madura foot) and only very rarely cryptococcosis and coccidioidomycosis.

### Incidence

The increased importance of systemic fungus infections in the past few years may be due to a real or an apparent rise in their incidence. The large numbers of patients with pulmonary aspergillosis now recognized may simply reflect a greater awareness of this condition. On the other hand, systemic candidiasis, cryptococcosis and histoplasmosis may be more frequently seen in this country because they occur as super-added infections in chronic disease, malignant disease and in patients receiving antibiotic or corticosteroid therapy. The accurate incidence of these conditions is not known. However, in the most highly endemic areas for coccidioidomycosis in the U.S.A. nearly 100 per cent of the population will have been infected in a few years and about one-fifth of these will have had an illness severe enough to cause temporary incapacity and to warrant medical care (Fiese, 1958).

Similarly, since it has been appreciated that histoplasmosis is not a uniformly fatal disease (Christie & Peterson, 1945), skin test studies have revealed a high incidence in endemic areas. In the United States of America histoplasmin sensitivity is highly prevalent in the east-central area, extending from the Mississippi to the Appalachian mountains and from the Great Lakes to the Gulf of Mexico. It is also found in Central America and some parts of South America. Inhabitants in certain localities within that area show as high as an 80 per cent incidence of positive reactions to histoplasmin skin tests (Edwards, 1958). Of individuals infected with *H. capsulatum* about three-quarters failed to show a clinical disease while the remainder had an influenza-like illness and of these 0.2 per cent developed a generalized infection.

Since *A. fumigatus* occurs in the sputum of about 10 per cent of bronchitic patients and in a higher proportion of asthmatic subjects (Riddell & Clayton, 1958; Pepys *et al.*, 1959), isolation by culture is not conclusive evidence of its pathogenic role. However, with improved methods and understanding of the diagnostic criteria of bronchopulmonary aspergillosis, this disease has been more reliably and more frequently recognized and 110 patients with bronchopulmonary aspergillosis were seen at the Brompton Hospital in a five-year period (Campbell & Clayton, 1964).

Cryptococcosis is of world-wide distribution and the saprophytic existence of *C. neoformans* in soil, now confirmed in various parts of the world, accounts for the sporadic cases all over the world. In Great Britain, 22 cases of cryptococcosis had been recorded by 1962 (Rook & Woods). The actual number of cases of this infection in this country is far higher, since single case reports of the disease are not regularly published.

Systemic infection with *Candida albicans* may affect the gastrointestinal tract, the heart, the bronchopulmonary system and the genito-urinary tract. The incidence of systemic candidiasis is difficult to assess but there is little doubt that the number of cases is rising because of the increasing importance of the predisposing factors which include antibiotic and corticosteroid therapy, the frequency of predisposing abdominal and gynaecological operations and the malignant lymphomas. Winner & Hurley (1964) stated that up to 1962, 20 cases of systemic candidiasis in children and 33 cases in adults had been reported in the literature.

Actinomycosis is of world-wide occurrence and is a relatively uncommon condition. Since the advent of penicillin therapy the chronic form of the disease associated with multiple discharging sinuses is rarely seen.

### ✓ Pathology

The tissue responses to infection with the deep fungi include most of the reactions known for bacterial, parasitic and other agents which cause inflammatory lesions. With the exception of *Sporotrichum schenckii*, the fungi causing the deep mycoses can usually be found in tissue sections, thereby allowing a positive diagnosis to be made. *Sporotrichum schenckii*, though rarely seen in local lesions, can be readily demonstrated in some of the lesions of the systemic forms of the disease. The tissue reaction to the fungi depends on the host's resistance and on the degree of hypersensitivity which is developed against the fungus by his cells. For example, in some individuals whose tissue cells develop no barriers to infection, *H. capsulatum* may grow abundantly within histiocytes, no localization takes place and spread throughout the body occurs causing disseminated fatal disease. In others, either due to natural resistance or due to a rapidly developing state of tissue hypersensitivity an epithelioid cell granulomatous reaction occurs promptly and this, in combination with the ensuing fibrosis, aids in confining the fungus and restrains dissemination (Binford, 1962).

*Actinomyces israelii* may produce an acute or chronic suppurative neutrophilic response. The typical "sulphur" granules may be easily seen with the hæmatoxylin and eosin stain, but the fungus itself is best demonstrated by a Gram stain or by the silver methenamine stain.

The most characteristic tissue reactions to deep fungus infection are the histiocytomycotic granulomatous reaction, pseudo-epitheliomatous hyperplasia, fibrosis, thrombotic arteritis, calcification, a mixed pyogenic and granulomatous histology and granuloma with caseous necrosis. In general, for histological staining of tissues for fungi the PAS reaction with Mayer's hæmalum as counterstain is valuable. Other staining techniques have proved of value in the detection of specific fungi (Riddell, 1963).

Only an outline of the mycological techniques required to make the diagnosis of a fungus infection can be given here. A clear and brief review of the steps involved in collection of specimens, their dispatch, direct and cultural examination of material and identification of fungi can be found in Broadsheet No. 43 of the Association of Clinical Pathologists (Riddell, 1962).

In general, infected material including pus, sputum, bronchoscopic aspirates, exudates, etc., should be examined as soon as possible and after minimal exposure to air. Microscopic examination of "wet" preparations is made in 10 per cent potassium hydroxide solution or with lactophenol blue or methylene blue-fuchsin stain. If cryptococcosis is suspected and particularly when CSF is being examined, a nigrosin or India-ink preparation is made to demonstrate capsules around the yeast cells. If actinomycosis is a possibility, pus and sputum are searched for white or cream-coloured granules. These are crushed and examined microscopically unstained and stained with Gram's and PAS stains. For histoplasmosis, Giemsa stain is useful for staining exudates and marrow smears. When pulmonary aspergillosis is suspected, the sputum should be carefully searched for "plugs"

or small bronchial casts, the number of eosinophil cells in the material should be estimated in smears fixed in alcohol-ether, stained by hæmatoxylin and eosin or by Gordon and Sweet's silver impregnation technique.

After microscopy, culture studies are carried out. If actinomycosis is suspected, plates of bacterial type medium (blood agar, heart-brain agar) without added antibiotics are heavily inoculated and incubated anaerobically and aerobically. For general routine culture of fungi, Sabouraud's medium (dextrose peptone agar) is inoculated heavily and incubated at 37°C. If dimorphic fungal infection is suspected, blood agar as well as Sabouraud's medium is inoculated and incubated at 22°C and 37°C. Bacterial growth can be suppressed by the incorporation of antibiotics (chloramphenicol or penicillin and streptomycin). Cycloheximide is added to inhibit contaminating fungi except when cryptococcosis, aspergillosis or mucormycosis are possible diagnoses.

TABLE 8.I

<i>Disease</i>	<i>Material for Direct Examination and Culture</i>	<i>Serological Tests</i>	<i>Skin Tests</i>	<i>Additional Investigations</i>
Actino- mycosis	Sputum/Pus.	No value.	No value.	
Nocardiosis	Sputum/Pus.	No value.	No value.	
Candidiasis	Sputum (thrush membrane if present). Fæces, urine or blood.	Doubtful value. (Precipitins in generalized infections.)	No value.	
Crypto- coccosis	Sputum/cerebro-spinal fluid. Bronchial and gastric washings. Aspiration biopsy material.	No value or doubtful.	No value.	
Aspergillosis				
1. Allergic	Sputum ("casts" or "plugs" especially examined).	Precipitins.	Prick test positive (*Aspergillus extract).	Blood or sputum eosinophils.
2. Mycetoma	Sputum.	Precipitins.	No value.	
Coccidioido- mycosis	Sputum, Bronchial aspirates. Gastric washings.	Agglutinins, precipitins and complement fixation test.	(†Coccidioidin.)	
Histo- plasmosis	Sputum, Bronchial aspirate. Gastric washings.	Agglutinins, precipitins and complement fixation test.	(†Histo-plasmin.)	Used in the same way as tuberculin in Mantoux test.
Blasto- mycosis	Exudates, Sputum.	Complement fixation test.	(†Blastomycin.)	
Sporo- trichosis	Pus or histology.	Precipitins may possibly prove of value.	Doubtful value.	

\* Bencard or Duncan and Flockhart.

† St. John's Hospital for Diseases of the Skin.

‡ Parke Davis.

Experimental animal inoculation may help in isolating scanty organisms in cases where cultures have failed or in assessing the virulence of isolated fungi. *Candida albicans*, for example, is lethal to rabbits within the week following intravenous inoculation of a standard quantity of yeast cells in saline. A virulent strain of *C. neoformans* causes death of white mice in two to four weeks after intraperitoneal inoculation of 0.5 ml of a 1 per cent suspension of packed cells in saline.

Serological tests and skin tests are helpful in the diagnosis of many systemic mycoses. Their diagnostic significance is summarized in Table 8.I (Clayton, 1966).

### Pathogenesis

There is a limited but growing amount of information about the factors which play a part in and predispose to the development of "opportunistic infections", particularly those caused by fungi. The superficial fungus infections are confined to the epidermis, and their affinity to the horny layer of the epidermis had in the past been thought to be due to keratinophilic properties of the dermatophytic fungi. However, Lorincz, Priestley & Jacobs (1958) showed that human serum contained a factor which had a fungistatic effect, and that this serum factor prevented growth of dermatophytes within a dialysis membrane suspended in the serum. This fungistatic effect of human serum was further demonstrated with the help of a miniaturized system for extracorporeal hæmodialysis in the dog (Goodman, Temple & Lorincz, 1961). This idea of an anti-fungal serum factor is not unique. It has recently been shown that a serum factor plays a part in combination with a platelet component, in acting as an antibacterial factor against *Bacillus subtilis* (Jago & Jacox, 1961).

Fungi responsible for the deep mycoses are also affected by factors present in cell-free human serum. *In vitro* studies of several of these fungal species have shown that human serum has an inhibitory effect on *Cryptococcus neoformans*, *Sporotrichum schenkii* and *Histoplasma capsulatum*. No difference was noted with penicillium, aspergillus and candida species, and enhancement of growth of *Blastomyces dermatitidis* was claimed (Baum & Artis, 1961). On the other hand, Roth & Goldstein (1961) demonstrated inhibition of *Candida albicans* by human serum, but found that this inhibition was markedly diminished in patients with acute blood dyscrasias.

Clearly, immunological factors also play a major part in the development of systemic fungus disease, and the experimental production of immunity has been investigated. Even antifungal vaccination might be a feasible practical step against the more common systemic mycoses in endemic areas, for example against coccidioidomycosis.

In candidiasis, in addition to clinical observations, there is a great deal of experimental evidence to show that a number of factors play a part in the pathogenesis of candidiasis. Such predisposing factors are antibacterial antibiotics, corticosteroids, neoplasia and operative procedures. Gynæcological operations, surgery on the heart and on the bowel may rarely be followed by systemic candidiasis or endocardial candidiasis. In *Candida* endocarditis in particular, the triad of damaged heart valves, administration of antibacterial antibiotics and trauma are almost inevitable aetiological agents.



These clinical observations are admirably documented by a controlled study on dogs. Experimental production of the lesions of *Candida* endocarditis was obtained in the following way: aortic regurgitation was surgically induced to 12 of 14 dogs. Several weeks later a large inoculum of *Candida guilliermondii* was injected intravenously into all the dogs including those in whom no valvular lesion was produced. Five of the dogs with induced valvular lesions were then given large daily doses of penicillin and streptomycin for eight days, four others with valvular lesions were given tetracycline for the same period. At autopsy, the dogs with normal valves or aortic regurgitation which were given *Candida* but no antibiotics showed no evidence of *Candida* endocarditis, whereas three of the five dogs with penicillin and streptomycin and one of the four with tetracycline therapy showed *Candida* endocarditis (Cooper *et al.*, 1961).

Four cases of systemic candidiasis following dental extraction were reviewed by Lehner (1964). He investigated the incidence of fungæmia following dental extractions and although blood samples taken after tooth extraction in 50 patients yielded *Streptococcus viridans* in 40 per cent of patients, no *Candida* or yeasts were isolated. On the other hand, saliva cultured from 50 patients grew yeasts in 42 per cent of patients. Lehner also showed that when normal blood was added to suspensions of *Candida*, consistent growth resulted only at concentrations above six cells per 4 ml of blood. He suspected that this finding cast doubt on the negative results in respect of *Candida* after extractions and he felt that a method which would eliminate the inhibitory activity of serum or in those with a deficiency of anti-candidal factor (as is found in patients with acute blood dyscrasias) post-extraction fungæmia might be detected.

### Radiological Features

Fungus infections of the lung and skeleton are likely to be diagnosed only if suspected, for there is often little specific in the radiological appearances. Almost any known pattern of lung pathology may be mimicked. For instance, the appearance may resemble tuberculosis in almost any of its forms from the primary infection through miliary tuberculosis to chronic fibrocaceous disease. At other times, the changes may resemble patchy localized or generalized bronchopneumonia, segmental or lobar pneumonia, suppurative pneumonia, chronic lung abscess, or empyema. In other cases there may be difficulty in distinguishing the mycotic infection radiologically from sarcoidosis or chronic interstitial pulmonary fibrosis. Hilar or mediastinal lymph node involvement is nearly always present and is an important diagnostic feature.

The X-ray changes seen most commonly in the various types of systemic fungus infection are described briefly in the text dealing with the individual disease entities. A summary of the radiological appearances is given in Table 8.II.

### ⚡ Therapy of Deep Mycoses

#### Amphotericin B

This drug is at present the first and the only available anti-fungal agent with activity against virtually all the systemic mycoses (Utz, 1965). Amphotericin A and B are produced by *Streptomyces nodosus* and they were dis-

	<i>Aspergillosis</i>	<i>Moniliasis</i>	<i>Actinomycosis</i>	<i>Histoplasmosis</i>	<i>Coccidioidomycosis</i>	<i>Blastomycosis</i>
1. Consolidation (a) segmental or (b) lobar	(a) or (b)	(a) or (b) slow resolving pneumonic consolidation	(a) or (b)	(a) may resemble pulmonary TB with "5" (b) +	(a) may resemble pulmonary TB with "5" (b) +	(a) or (b)
(c) transient shadows	(c) allergic type	—	—	—	—	—
2. Miliary shadows (a) fine (b) coarse	(a) + with "5" and "6" to resemble sarcoid (b) +	(a) (b) occasional	(b) +	(a) + (b) + +	(a) + (b) + +	(b) +
3. Cavitation	Mycetoma may form in pre- existing cavity—in upper lobe, or apex lower lobe + —	Mycetoma occasional	Common. Thick or ragged walls	Unusual but with "6" may look very like chronic TB	Common. (a) small "doughnut" (b) thin walled tension cavity + +	Common. As with Actinomycosis + —
4. Hilar and/or mediastinal lymph node enlargement	See "1". When fine and generalized may resemble chronic interstitial fibrosis	Interstitial	Local. Coarse	May be only abnormality —	—	Local. Coarse
5. Fibrosis	—	Occasional	Usual. Common	+	—	Usual
6. Pleural involve- ment. Empyema	—	—	Ribs, common. Spine, occasional. Jaw.	—	+	+ Ribs, common. Any part of skeleton
7. Bone involvement	Rare	—	—	Dense miliary all zones. May be "halo". Hilar nodes Any area. Unilateral or bilateral	Miliary or solitary Hilar nodes	—
8. Calcification	Any area. Unilateral or bilateral. Occa- sionally generalized	Any area.	Any area but more often basal	—	Any area Unilateral or bilateral	Any area.
9. Lung region affected	—	—	+	—	—	+
10. Abscess of chest wall	—	—	—	—	—	—

covered in 1956. Detailed animal studies showed that Amphotericin B was more active than Amphotericin A and it was found more suitable for systemic use in man. The exact chemical composition of Amphotericin B has not been defined but, as its name suggests, it is an amphoteric substance—soluble at high or low pH in water or in aqueous alcohols. Amphotericin B is poorly absorbed from the gastrointestinal tract and, following oral administration, little or none is detected in the serum. This antibiotic is active *in vitro* against the yeast and yeast-like fungi, most organisms causing the deep human mycoses and strains of *Leishmania brasiliensis* and *L. donovani*. The exact mode of action of Amphotericin B is not known, but it has been suggested that like Nystatin, it may alter the permeability of the cell wall to small ions. *In vitro*, *Candida* species have been shown capable of developing resistant strains to Amphotericin B but this complication has not been reported in clinical practice. Newcomer and co-workers (1960) found that growth of the following fungi was inhibited over a period of 10 days in the presence of 0.5 mcg/ml. of Amphotericin B: *Coccidioides immitis*, *Candida albicans*, *Cryptococcus neoformans*, *Blastomyces dermatitidis*, *Histoplasma capsulatum*, *Trichophyton rubrum* and *Microsporum audouinii*.

**Modes of Administration of Amphotericin B in Man.** Because of the poor solubility of the drug in aqueous fluids and consequently its inadequate absorption after oral administration, an Amphotericin B complex with sodium desoxycholate has been produced with improved solubility. This drug combination has an increased activity, but it is too toxic when given by mouth and is more toxic than the parent drug given intravenously.

On the basis of published reports (Utz, 1965) oral administration of Amphotericin B has been followed by healing of lesions due to *Blastomyces dermatitidis*, *Cryptococcus neoformans* and *Coccidioides immitis*. However, intravenous administration of the drug is indicated in all patients with cryptococcal meningitis, severe disseminated and the more chronic forms of cavitary pulmonary histoplasmosis, severe disseminated coccidioidomycosis, disseminated aspergillosis and *Candida* septicæmia, endocarditis or meningitis. It is of value in North American blastomycosis, pulmonary cryptococcosis and acute primary histoplasmosis. It has also been claimed to be useful in phycomycosis, iodide-resistant sporotrichosis and South American blastomycosis. The drug has been used intrathecally at lumbar, cisternal or intraventricular sites in patients with cryptococcal or coccidioidal meningitis. Amphotericin B has been used by injection into the anterior chamber of the eye, into the joint or thoracic cavity and locally injected into skin lesions, e.g. in chromoblastomycosis.

Aerosol therapy in pulmonary mycoses has been advocated and the drug can be used topically in cutaneous candidiasis.

**Dose of Amphotericin B.** The intravenous route of administering Amphotericin B is the method of choice. There is as yet no accepted standard dosage scheme nor is there complete agreement as to the total dose of the drug and the duration of the treatment. The drug is put up as a sterile powder consisting of 50 mg of Amphotericin B with 41 mg of sodium desoxycholate and some sodium phosphate buffer. This is used in a 5 per cent aqueous glucose solution which is suitable for intravenous and intrathecal administration:

Of the many recommended dosage schemes only the following will be

described in detail. An initial dosage of 10 mg of Amphotericin B is given the first day, 25 mg the second day and 50 mg each subsequent day (Hildick-Smith, Blank & Sarkany, 1964) in 1,000 ml. of 5 per cent glucose over a six to eight hour period. Side effects are treated symptomatically and 10 mg of heparin are added to reduce the tendency to local vein thrombosis. Salts such as sodium chloride and procaine hydrochloride when introduced into the infusion fluid will cause precipitation of the amphoteric antibiotic and should be avoided.

Utz (1965) prefers an initial dose of 1.0 mg of the drug. The dose is increased by daily increments of 5 mg until an optimal dose of 0.5 to 1.5 mg/kg/day is reached. Both the rate of increase and the optimal dose are governed by the degree of toxicity. The daily amount of the drug is diluted in 500–1,000 ml. of 5 per cent glucose in water and given over a 2–6-hour period. The bottle is shielded from light during the infusion. In children, Little and co-workers (1959) recommend 1 mg in 10 ml solution over a period of six hours. The initial dose should correspond to 0.25 mg/kg and the daily dose is then increased 1–2 mg each day or every other day until a maximally tolerated dose is achieved which is usually of the order of 1 mg/kg.

At least a total of 1 g of the drug and one month of therapy are accepted as a minimal schedule. In adults a total dose of up to 5 g appears to be safe and any impairment of renal function following an intravenous dose of this order is usually reversible. A total dose of 5–10 g and over is associated with the risk of permanent impairment of renal function.

Oral administration of Amphotericin B is of little or no value in the treatment of deep mycoses, but it has been used in treating intestinal candidiasis. It has also been suggested that in combination with tetracycline or its congeners Amphotericin B suppresses the proliferation of yeasts in the gut which may be associated with the oral use of these antibacterial antibiotics.

There is a high renal threshold for Amphotericin B excretion and consequently slow urinary excretion and relatively long-maintained blood levels. The serum levels which have been reported differ with the methods employed and the maximum blood levels range from 0.3–3.65  $\mu\text{g/ml}$ , rarely exceeding 1.5  $\mu\text{g/ml}$ . Significant activity can be detected after 24–48 hours. The minimal inhibitory concentration of most fungi causing the systemic fungus infections are well within the range of the blood levels resulting from intravenous administration of Amphotericin B.

Since renal function impairment is the most significant side-effect of Amphotericin B therapy, regular examination of the urine is essential. It has been suggested that the creatinine clearance test of renal function is a useful investigation which should be carried out at frequent intervals. The blood count, serum potassium levels and liver function tests (serum bilirubin, SGOT and alkaline phosphatase) should also be done, preferably at weekly intervals during the course of Amphotericin B therapy.

The common side-effects of intravenous Amphotericin B administration are nausea, anorexia, vomiting, fever, chills, rigors, headache and malaise. Chlorpromazine given orally 30 minutes before the start of the infusion or intravenously administered hydrocortisone during the course of intravenous Amphotericin B therapy have been claimed to reduce significantly the incidence of side-effects.

**X-5079 C**

X-5079 C is a polypeptide antifungal antibiotic which has been shown to be effective in patients with histoplasmosis, sporotrichosis, North American blastomycosis and to a lesser degree in aspergillosis and maduromycosis. Six-hourly subcutaneous injections of 3–17 mg/kg/day for periods of 7–70 days have been advocated. Side-effects in man are mainly confined to impairment of liver function, as shown by abnormal sulfobromophthalein excretion, by occasional increase in the conjugated or unconjugated serum bilirubin and in a small proportion a raised serum aspartate transaminase and alkaline phosphatase.

There is inadequate information concerning this drug, but in spite of the high relapse rate following its use and impairment of liver function, it promises to become a useful adjunct to therapy of systemic mycoses.

**Hamycin**

Hamycin is a new antifungal antibiotic which belongs to the polyene group, it is derived from *Streptomyces pimprina* and is a product of India. In superficial fungus infections in man, it was found to be mainly useful against *Candida* infections (Sarkany & Caron, 1966), but in experimental studies in mice (Bennet *et al.*, 1964) it had a striking effect against brain infections by *Cryptococcus neoformans*. Studies in human systemic mycoses are in progress.

**Pimaricin**

Pimaricin is a tetræne antifungal antibiotic with *in vitro* activity against a wide range of fungi. It has been used with effect topically and is relatively non-toxic. Used as an aerosol in a small series of patients with bronchopulmonary aspergillosis pimaricin was reported to be useful (Edwards & La Touche, 1964).

✓ **Summary of Therapy of Systemic Mycoses**

Amphotericin B is clearly the single most important specific therapeutic agent in systemic fungus infections. It is the standard to which other drugs must be compared. Unfortunately, however, the side-effects of this drug are sufficiently severe to justify continuing search for new agents. Equally some of the older preparations effective in systemic mycoses continue to be used and remain the drug of choice in some conditions. Potassium iodide in sporotrichosis, penicillin and sulphonamides in nocardiosis and actinomycosis are still drugs of first choice and 2-hydroxystilbamidine is advocated by some in North American blastomycosis. Table 8.III summarizes the choice of therapeutic agents used systemically in deep fungus infections and is a substantially modified version of one published elsewhere (Medical Letter, 1965).

TABLE 8.III

## SUMMARY OF THERAPEUTIC AGENTS IN SYSTEMIC MYCOSES

<i>Disease</i>	<i>Drug of First Choice</i>	<i>Alternative Drugs</i>
Actinomycosis	A penicillin	Tetracycline Chloramphenicol
Nocardiosis	Sulphonamides	Tetracycline Chloramphenicol Streptomycin
Histoplasmosis	Amphotericin B	A sulphonamide
Candidiasis Pulmonary Candidiasis Systemic	Nystatin Amphotericin B	} Pimaricin
Aspergillosis (allergic type)	Nystatin inhalations	
Coccidioidomycosis	Amphotericin B	No dependable alternative
Blastomycosis North American South American	Amphotericin B Amphotericin B	2-hydroxystilbamidine Sulphadiazine
Sporotrichosis	Iodides	Amphotericin B

## ACTINOMYCOSIS

Actinomycosis is a chronic, granulomatous, pyogenic infection caused by *Actinomyces israelii*, an anaerobic Gram-positive branching filamentous actinomycete. This organism is more closely related to bacteria than to fungi. The infection is of endogenous origin and the responsible organism, *Actinomyces israelii*, has never been found free in nature but is thought to exist as a saprophyte in the tonsils and in the roots of carious teeth. The trauma to tissue associated with dental extraction or even from biting is believed to allow the organism to act as a pathogen and produce the disease in the cervico-facial region whereas aspiration or swallowing of the organism probably result in the pulmonary or abdominal form of the disease. In all these sites the disease starts as an inflammatory lesion which slowly goes on to abscess formation with pus containing "sulphur granules" in which the "ray fungus" is found. Actinomycosis is of world-wide distribution.

## Clinical Features

Of the three major sites involved, the cervico-facial area is most common (56.8 per cent), the lungs are affected in 22.3 per cent and the abdomen in 15 per cent of cases (Cope, 1938).

**Cervico-facial Type.** Cervico-facial actinomycosis frequently follows 2-3 months after a dental extraction. The lesion presents as a swelling over the mandible or the side of the face, increases to form a brawny mass, breaks down and discharges pus containing "sulphur granules". In the advanced stages, multiple sinuses form leading from the granuloma to the surface. The regional lymph nodes are characteristically not enlarged, an important point in differential diagnosis. The advanced stage of the disease with multiple sinuses has now become rare because of early diagnosis (often by dental surgeons who are particularly familiar with the picture) and effective treatment. Five patients with cervico-facial actinomycosis related to periodontal disease or trauma, confirmed by isolation of the organism and treated early were recently reported (Caron & Sarkany, 1964).

**Pulmonary Form.** This may be unilateral but may become bilateral. The onset of the disease (Riddell, 1963) is usually insidious, with slight and irregular fever, cough and expectoration. When suppuration develops, the sputum becomes muco-purulent and sometimes bloodstained. The clinical picture may resemble tuberculosis, lung abscess or a necrotizing new growth. Pleural pain, evidence of consolidation and mediastinal involvement may occur, but occasionally a chronic empyema containing foul-smelling pus may develop (Bowyer, 1949). The disease spreads across fascial planes and eventually abscesses and sinuses in the chest wall develop. Bates & Cruickshank (1957) studied 85 cases of thoracic actinomycosis and suggested a classification under the following headings:

- (a) Primary (pleural, pulmonary, lung abscess, mediastinal).
- (b) Secondary (extension from abdominal or cervico-facial actinomycosis).
- (c) Metastatic and actinomycosis associated with pulmonary tuberculosis.

They considered that infection from the tonsils was an important ætiological factor in the younger age group and dental sepsis in the older. Secondary extension from abdominal actinomycosis was the second most common cause of infection in the chest in this series.

A case of actinomycosis of the lung with secondary involvement of the spine was described by Young (1960). When the spine is involved, several successive vertebræ are affected and there is nearly always evidence of a paravertebral abscess.

X-rays show irregular shadows, sometimes homogeneous opacities, suggesting consolidation or pleural effusion. Periostitis and osteitis of the ribs may be seen in radiographs as a result of direct extension of infection from soft tissues. A characteristic X-ray finding is new bone formation on the undersurface of contiguous ribs. In more chronic cases, especially when associated with secondary infection, this periosteal reaction gives rise to thickening of the ribs (Fig. 8.1). Vertebral collapse is not uncommon but the discs are usually not involved.

**Abdominal Actinomycosis.** *Actinomycoses israelii* may invade the cæcum, appendix and other abdominal structures and may produce granulomatous lesions in these, giving the symptoms and signs referable to the individual organs. An irregular indefinite mass may be felt in the ileocecal region or elsewhere in the abdomen. At this stage, the correct diagnosis is usually made following laparotomy and subsequent histological and bacteriological

examination. In the later phases of the disease, fever, vomiting, intestinal colic and weight loss may be noted. Widespread involvement of the abdominal structures, including bone results and sinuses with the characteristic pus containing "sulphur granules" appear. Abscesses are a characteristic feature of actinomycosis. Sinuses may follow spontaneous rupture or incision of an abscess, a laparotomy or an appendectomy and they are said to point laterally or posteriorly. Infection of the vertebral bodies or a psoas abscess may result. In contrast to tuberculosis, where only the anterior portion of the vertebral bodies is radiologically involved, periostitis with erosion of cortical bone, destruction of the laminæ and vertebral processes is seen.

### ✓ Treatment

Penicillin continues to be the drug of choice against actinomycosis. A six-weeks course of 1–2 million units daily is effective in early diagnosed cases of cervico-facial disease, but up to 10–12 million units daily may be required for up to 6–8 months in advanced cases of the pulmonary or abdominal form. If the response to penicillin is inadequate or the patient is sensitive to penicillin, streptomycin or another antibiotic should be chosen, preferably selected on the basis of *in vitro* sensitivity tests. In general, surgery rarely has any part to play even in the treatment of thoracic actinomycosis (Tubbs, 1958). Chronic sinuses and small abscesses in the chest wall no longer require surgery, since they respond to penicillin. However, occasionally a great deal of bronchiectasis or fibrosis may develop due to secondary infection and surgery may be indicated for this type of structural damage.

## NOCARDIOSIS

Nocardiosis is a chronic progressive infection of the lungs which occasionally affects the central nervous system and the skin. It is of world-wide distribution, forms about 10 per cent of infections due to the actinomycetes and is caused by *Nocardia asteroides*—an aerobic, Gram-positive and partially acid-fast organism. In contrast to *Actinomyces israelii*, *Nocardia asteroides* is an organism found in the soil and may be introduced into the tissues of man as a result of injury or inhalation. For this reason, it is seen more commonly in agricultural workers.

Pulmonary nocardiosis clinically resembles tuberculosis and is characterized in the early stages by cough, dyspnoea, malaise, fever and hæmoptysis. Because of the great variety of lung lesions, e.g. lung abscess, pulmonary mycetoma, pneumonia, fibrosis and infection of the pleura or chest wall, nocardiosis may have to be differentiated from tuberculosis, actinomycosis, new growths, pyogenic lung abscess and other fungus diseases of the lung. Accurate diagnosis is vital because of the specific response of the disease to therapy. The presence of nocardial branching filaments in the sputum or in areas of suppuration and necrosis and culture of cream to orange colonies under aerobic conditions helps to separate the disease from tuberculosis and the other conditions mentioned.

Nocardial infection, like that caused by a number of other opportunistic fungi, tends to be associated with lymphomas and other diseases which impair the host-immune mechanisms (Hildick-Smith, Blank & Sarkany, 1964), e.g. progressive chronic disease, leukæmia, Hodgkin's disease, diabetes



mellitus, alveolar proteinosis, Cushing's syndrome as well as with the use of corticosteroids, antibiotics and cancer chemotherapeutic agents.

This is a very serious systemic mycosis with a very high mortality rate. Sulphonamides (e.g. sulphadiazine) are the chemotherapeutic agent of choice. High dosage and a prolonged treatment are required. Combination with other antibiotics may be necessary, the choice being based on *in vitro* sensitivity data. This form of treatment should be combined with surgical excision or drainage of suitable lesions.

### CANDIDIASIS

*Candida albicans* occurs both in the shape of rounded yeast-like organisms and in a mycelial form. Essentially, the former is saprophytic and it may undergo a change to the mycelial or pathogenic form. What exactly determines the development of the particular form of this organism is not clearly understood, although, *in vitro*, certain nutritional factors tend to favour the transformation of yeast into mycelial forms. It is likely that *in vivo* environmental factors which have a bearing on the metabolism of the cell, encourage such transformation. *Candida* species, including *C. albicans*, are found in various sites of the human body as harmless saprophytes and their presence in the mouth, the gastro-intestinal tract (stools), the sputum, the vagina, on the perianal skin and in other sites does not by itself signify disease. In discussing the factors which predispose to the development of thrush, Winner & Hurley (1964) tabulated the "abnormal states" as follows:

1. Physiological: pregnancy, infancy.
2. Local trauma, maceration, allergy of skin.
3. Disorders of the endocrine system: diabetes mellitus, hypoparathyroidism, Addison's disease, pancreatitis, hypothyroidism.
4. Malnutrition.
5. Malabsorption syndrome.
6. Antibiotic and steroid therapy.
7. Blood dyscrasias, in particular acute leukæmia, agranulocytosis, and aplastic anæmia.
8. Post-operative states.
9. Malignant disease.

Essentially, the same factors operate in the production of systemic *Candida* infection. In an evaluation of the fungistatic activity of serum (Roth *et al.*, 1959; Roth & Goldstein, 1961), 5–20 per cent concentrations of human serum added to a nutrient medium inhibited the growth of *Candida in vitro*. Serum of infants under the age of three months, from patients with acute leukæmias, terminal chronic leukæmias, Hodgkin's disease, multiple myeloma and erythemic myelosis showed a lowered titre of the anti-*Candida* component. There is also a great deal of information on the presence of *Candida* antibodies in the serum. Unfortunately, however, neither agglutinins, complement fixing antibodies nor precipitins are of diagnostic significance, since the former may be present in many people who have no overt clinical infection and a high titre of the latter may be often present in the general population (Winner & Hurley, 1964).

### Systemic Candidiasis

Candidiasis may occur as a generalized septicæmic acute disease or it may selectively affect certain systems of the body, particularly the gastro-intestinal tract, the kidneys, the lungs, the central nervous system or the heart. Winner & Hurley's (1964) review of acute disseminated septicæmic candidiasis deals separately with *Candida* septicæmia in children and in adults. The main reason for the separation into these two groups was the difference in prognosis—many of the children recovered, the adults died almost invariably.

Up to the middle of 1962, they were able to trace and accept as proven only 16 cases in children in whom the diagnosis had been substantiated by culture of the organism from the blood stream during life or from the mycotic lesions at necropsy. In all cases, except one, the child had been previously treated with at least one but more often with multiple antibiotics. The associated disease in these children ranged from urinary and chest infections to ear infections, gastroenteritis, lipid nephrosis. *Candida* species were cultured from the blood stream and in one child also from the cerebrospinal fluid and the urine. Clinical details were inadequate but pyrexia, thrush and skin lesions were amongst the initial changes. Six of this series of children recovered without specific anti-*Candida* antibiotic therapy. It would appear that transient *Candida* septicæmia may occur in children without necessarily producing irreversible damage to organs.

In their analysis of 28 cases of systemic candidiasis in adults, the septicæmia was preceded in the majority by operations, blood dyscrasias, antibiotic or steroid therapy.

In the fatal cases of systemic candidiasis, *C. albicans*, *C. tropicalis* or *C. pseudotropicalis* were cultured from the blood stream or from the organs at necropsy. Fever, thrush, ulceration of the mouth and gums, "dermatitis" and central nervous system involvement giving rise to a "depressed sensorium", i.e. a state of diminished response to external stimuli, were the main clinical features. Other symptoms and signs depended on involvement of particular organs.

Post-mortem findings in the adults consisted of widespread fungus infection, the main sites of involvement being the kidneys, less often the brain, liver, lungs, blood vessels, spleen and thyroid. In the kidney, there were areas in the cortex, less often in the medulla, consisting of fungus in the mycelial and yeast-like phase. These were occasionally surrounded by polymorphonuclears and they were sometimes large enough to be seen with the naked eye.

Some of the children with systemic candidiasis recovered spontaneously. Only few adults recovered—all these had been treated with Amphotericin B.

### Candidal Infection of the Intestinal Tract

**Oral Candidiasis.** Oral candidiasis or thrush may occur in persons of all ages, but is much more common in infants and chronically ill adults. The incidence of thrush in the newborn has been estimated at between 4 and 10 per cent. Recent work has shown that thrush in the newborn is related to the presence of *Candida* in the maternal birth canal and that it is not due to cross infection.

The infection may affect the buccal mucous membranes, the tongue, the gums and less often the palate, fauces and tonsils. It may spread to the œsophagus and trachea. It may also occur, particularly in older edentulous people, at the corners of the mouth. This is usually due to faulty dentures, a diminution of the vertical dimension of the lower third of the face and exaggerated apposition and sagging of the oral commissural folds. Small greyish white patches surrounded by a slight erythematous halo may gradually extend into large continuous plaques. These consist of friable material which, when scraped off the underlying mucosa, leaves a moist bleeding surface.

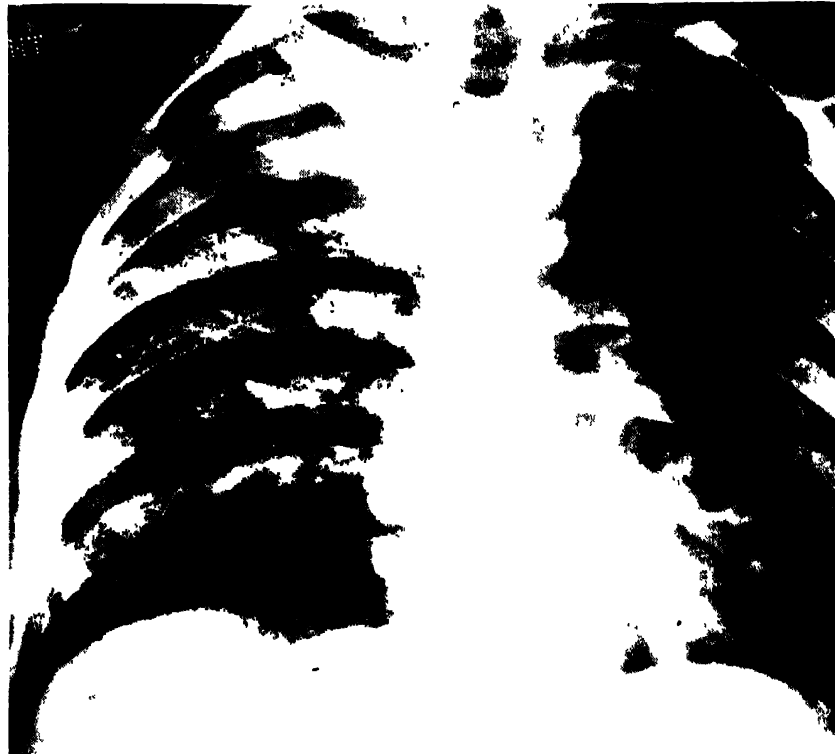
In the treatment of oral candidiasis, 1 per cent gentian violet has now been supplanted by anti-fungal antibiotics, particularly Nystatin and Amphotericin B. One to four ml of Nystatin (100,000 units per ml) in aqueous suspension 4–6 times daily for 7–14 days is almost invariably effective in infants and controls the infection in adults. The suspension is put directly into the mouth and is not painted on with a cotton wool applicator. Prophylactic applications in the newborn have been claimed to prevent infection by *Candida*.

**Candida Infection of the Œsophagus and the Intestine.** In œsophagitis due to *C. albicans* there is almost invariably oropharyngeal candidiasis. The condition is rare both in infants and adults and is not infrequently diagnosed post-mortem only. In adults it may occur in association with severe debilitating disease and it has been claimed to have followed the use of the tetracyclines and systemic steroids. Characteristic radiographic changes due to candidiasis of the œsophagus in a woman with systemic lupus erythematosus after prolonged treatment with ACTH were reported by Hogewind & Hogewind (1957). Similar X-ray changes were noted by Young (1966) (Fig. 8.2).

The clue to *Candida* œsophagitis in infants is the presence of *Candida* infection elsewhere, particularly in the mouth and pharynx. Vomiting, choking, a tendency to cyanosis during feeding, other feeding difficulties and consequent failure to thrive, dehydration and toxæmia are suggestive. Numerous mycelial forms on direct examination, a positive culture and a therapeutic response to anti-*Candida* agents are suggestive or confirmatory signs in the diagnosis.

In infants there is associated thrush and usually also candidiasis of the lungs. In adults, there may be dysphagia and substernal pain and the diagnosis may be made or confirmed by œsophagoscopy.

The incidence and frequency of *Candida* enteritis is still undecided. The fact that this organism may be recovered from the normal gut adds to the difficulties of establishing the diagnosis. However, usually the yeast form only is present and the presence of hyphal elements always suggests that *Candida* plays an ætiological role in the causation of the enteritis. Actual histological invasion of the gut mucosa is diagnostic of *Candida* enteritis, but such confirmation can only rarely be obtained during life. There has undoubtedly been an increase in the incidence of this condition during recent years, following the use of broad-spectrum antibiotics, although the disease is still rare, if strict diagnostic criteria are observed. Transient diarrhœa following broad-spectrum antibiotics is not uncommon and is occasionally blamed on *Candida* enteritis. However, Brabander, Blank & Butas (1957) pointed out



**FIG. 8.1. PULMONARY ACTINOMYCOSIS.**

Ill-defined shadowing throughout the greater part of the right lung with thickening of the pleura on the lateral chest wall and in the minor fissure. There is periosteal new bone formation on the posterior ends of the 3rd, 4th, 5th and 6th ribs. (By courtesy of Dr G. Simon.)



FIG. 8.3. PULMONARY CANDIDIASIS.

Extensive miliary type involvement of lungs. There is some enlargement of the hilar shadows. The X-ray features are not distinctive. (Reproduced from "Fungus Diseases and their Treatment": G. Hildick-Smith, H. Blank and I. Sarkany (1964), London, Churchill.)

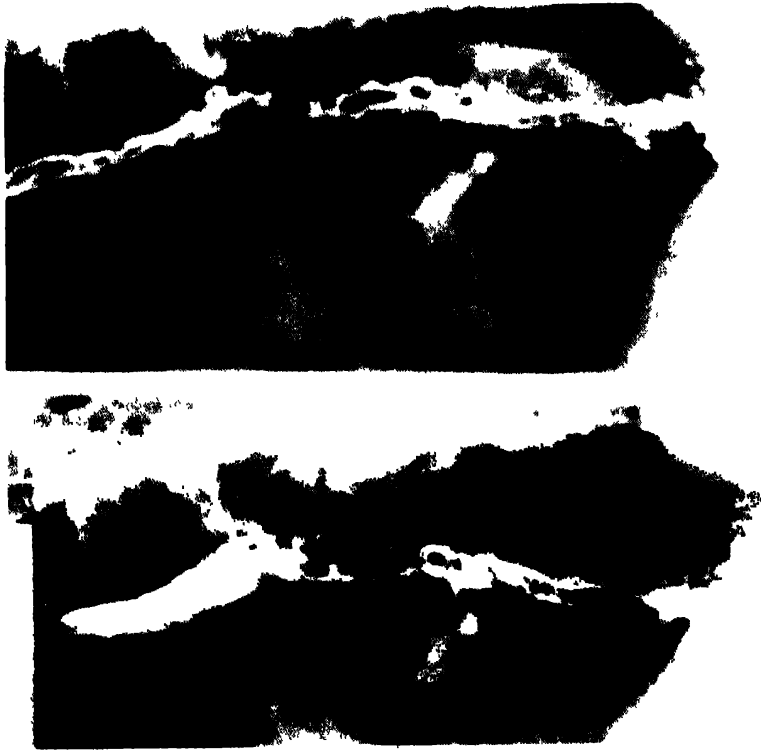


FIG. 8.2. CANDIDIASIS OF THE OESOPHAGUS.

Barium swallow showing candida oesophagitis in a patient treated with antibiotics. The filling defects are somewhat suggestive of oesophageal varices but the pattern is different and there is more extensive involvement. (By courtesy of Dr W. B. Young.)



FIG. 84. ASPERGILLOSIS ASPERGILLOMA.

Large aspergilloma almost filling tuberculous cavity in the right apex. The sputum contained *Aspergillus fumigatus*. A right upper lobectomy revealed a typical pink fleshy mass in the cavity. (By courtesy of Dr G. Simon.)

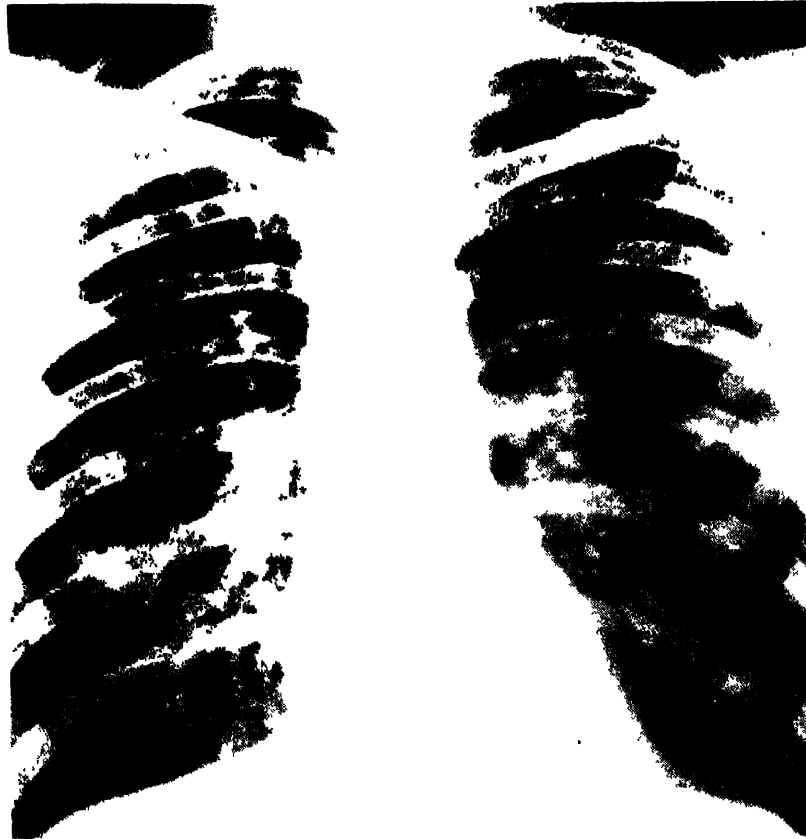


FIG. 8.5. ASPERGILLOSIS--ALLERGIC TYPE.

Chest radiograph of a man aged 24 years with a 6 years' history of productive cough and wheezing which started after threshing wheat.

*Aspergillus fumigatus* was grown from the sputum and a skin test with aspergillus extract was positive. The white cell count was 11,000/c.mm. 22 per cent eosinophils. Note enlarged hilar nodes, ill-defined opacities throughout both lung fields with accentuation of linear markings at both bases. (By courtesy of Dr. G. Simon.)



FIG. 8.6. COCCIDIOIDOMYCOSIS OF BONE.  
Destructive lesion with surrounding sclerosis in outer end of right clavicle.



FIG. 8.7. PULMONARY COCCIDIOIDOMYCOSIS.  
Ill-defined consolidation in region of left hilum with a little patchy shadowing  
in right mid-zone.  
(Both illustrations are from "Fungus Diseases and their Treatment". G. Hildick-  
Smith, H. Blank and I. Sarkany (1964), London, Churchill.)





FIG. 8.8. PULMONARY COCCIDIOIDOMYCOSIS.

Lateral view following induced pneumoperitoneum shows well-defined cavity with fluid level in apex of lower lobe. This is considered to be characteristic of *Coccidioides immitis* infection.

(Both illustrations are reproduced from "Fungus Diseases and their Treatment". G. Hildick-Smith, H. Blank and I. Sarkany (1964). London, Churchill.)



FIG. 8.9. COCCIDIOIDOMYCOSIS.

Surgical specimen of lung which has been bisected, revealing a cavity similar to that in Fig. 8.8.



FIG. 8.10. PULMONARY HISTOPLASMOSIS.

Dense scattered calcified lesions throughout both lung fields and in hilar nodes due to previous infection. There are some residual fibrotic changes in the left middle and both lower zones. (By courtesy of Dr W. B. Young.)



FIG. 8.11. SPOROTRICHOSIS.

The lymphangitic form with nodules up the leg. (Reproduced from "Fungus Diseases and their Treatment". G. Hildick-Smith, H. Blank and I. Sarkany (1964), London, Churchill.)

that only an increase in bowel movements or recurrent attacks of diarrhoea, occasional rectal bleeding, abdominal discomfort, rectal pain and pruritus associated with the regular recovery of significant amounts of *C. albicans* from the stools, in the absence of any other demonstrable cause, were acceptable features for making the diagnosis. If, in addition, the symptomatology disappeared following the administration of anti-Candida agents and the yeasts were eliminated from the stools, the diagnosis could be considered proved.

Pruritus ani, with or without diarrhoea following the use of antibacterial antibiotics, does not by itself denote Candida enteritis. In many cases, the irritation and possibly the loose stools may be due to a direct chemical irritant effect of the antibiotic; in others, they may be caused by the altered flora and the overgrowth by organisms resistant to the antibiotic. Yet in others, an overgrowth by pathogenic species of *C. albicans* may be responsible.

Candida oesophagitis in adults has been successfully treated with troches each containing 100,000 units of Nystatin and allowed to dissolve in the mouth at 2-4-hourly intervals (Lees, 1959). In infants, 100,000 units of Nystatin in aqueous solution at 2-hourly intervals is effective. Supportive measures and proper hydration are essential.

Candida enteritis in adults and in infants has been successfully treated with oral Nystatin and oral Amphotericin B. Nystatin in a dose of 1-2 million units daily over 7-10 days has been found curative in Candida enteritis in adults.

**Candida Endocarditis.** This rare form of endocarditis has clinically the characteristics of endocarditis of the bacterial type but the causative organism cultured from the blood and producing vegetations on the heart valves is one of the pathogenic species of Candida, predominantly *C. albicans*. Previously diseased heart valves are a prerequisite, but not invariable. Introduction of the fungus into the circulation may occur during operations on the heart, mainly valvotomies, but abdominal and gynaecological surgery, intravenous therapy or intravenous injections, whether therapeutic or by narcotic addicts, may be responsible. The recent increase in heart surgery has led to a rise in incidence of this almost universally fatal type of endocarditis. Another predisposing factor is the administration of broad-spectrum antibiotics.

Clinically, pyrexia, malaise, abdominal pain, nausea, vomiting, hepatomegaly and heart failure in a patient with old valvular disease are features which resemble those of bacterial endocarditis. A specific feature of Candida endocarditis stressed by many authors is evidence of embolism in the large arteries. At operation or at autopsy, there are massive vegetations on the heart valves. These large friable vegetations consist of masses of fungal elements and are probably one of the main reasons for the extremely bad prognosis in this disease. Amphotericin B intravenously in the highest tolerated dosage is the only potentially curative form of treatment. Recently this has been combined with surgical removal of the vegetations. There have now been several patients who have recovered through this combined therapeutic approach.

**Pulmonary Candidiasis.** The exact frequency of lung disease due to *C. albicans* is difficult to establish. Most authors agree that genuine candidal

bronchopulmonary infection is rare and many cast doubt on the existence of candidal asthma and pneumonia, although well authenticated case reports of both exist. The main diagnostic difficulty arises because *C. albicans* is a normal inhabitant of the respiratory tract and is recovered on culture from the sputum of approximately 55 per cent of patients with pulmonary tuberculosis, 27 per cent of other hospitalized patients and from approximately 13.5 per cent of healthy individuals.

Predisposing factors to pulmonary candidiasis are treatment with antibiotics, systemic corticosteroids and underlying pulmonary disease, especially pulmonary tuberculosis, bronchiectasis, carcinoma, pulmonary silicosis and asthma.

Occasionally, bronchopulmonary infection, asthma or pneumonia are diagnosed simply on the basis of repeated positive culture of *C. albicans*. This in itself is not justified and may be misleading. Any of the above mentioned underlying diseases may be masked by the Candidal superinfection and therefore missed. Finally, lung disease due to antibiotic resistant organism has to be ruled out. When repeated positive sputum cultures are found or bronchopulmonary washings yield on successive occasions *C. albicans* and the patient is suffering from a severe febrile illness with a background of debilitating disease, the diagnosis of pulmonary moniliasis may be accepted.

The clinical and radiological findings have been summarized by Riddell (1963):

**Bronchial Candidiasis.** The symptoms and signs are essentially those of bronchitis. Cough, associated with scanty sputum, sometimes mucoid, occasionally milky in appearance, may contain small grey flakes in which fungus is present. X-rays may show faint ill-defined patchy shadows, sometimes with hazy linear streaking mainly in the mid-zone and basal areas.

**Pulmonary Candidiasis.** The cough is distressing and the sputum may be bloodstained. Dyspnoea and pain in the chest may be the presenting symptoms. In contrast to bronchial moniliasis, the patient is ill, pyrexial, with a raised respiration and pulse rate. Consolidation and pleural effusion may be present. X-rays show ill-defined patchy shadowing, usually with sparing of the apices (Fig. 8.3).

The sputum contains both budding yeast cells and filaments of *Candida*. These are present particularly in any fragments of thrush membrane which may be coughed up and which has separated from the bronchial wall.

Aerosols containing gentian violet or brilliant green have been used and oral administration of iodides, Nystatin and Amphotericin B has been tried with little success. Poor absorption of the last two from the gastro-intestinal tract is probably the reason for their failure. Nystatin given in aerosol form every 4–6 hours is effective. In cases of pulmonary candidiasis combined with candidal involvement of other organs intravenous Amphotericin B therapy is indicated.

**Candidiasis of the Urinary Tract.** When small doses of *C. albicans* are injected intravenously in a mouse (Hurley & Winner, 1963), only the kidneys are affected, the other organs being spared. While human urinary tract candidiasis may be part of systemic septicæmic involvement, renal candidiasis may equally be a separate entity, although both types of disease are fortunately

rare. It has been suggested that, at least in some women, the yeasts are transferred to the urinary tract from the vagina. The condition is more common in women.

Candidal infection of the urinary bladder is more common than pyelonephritis due to this fungus. The symptoms are exactly similar to those met with in cystitis of bacterial ætiology, but a plaque of "thrush" may be seen on cystoscopy. Repeatedly positive cultures of the urine for *C. albicans* in the absence of another demonstrable cause are confirmatory evidence. Pyelonephritis due to *C. albicans* may be associated with renal calculi and may affect the function of the kidneys.

In cystitis due to *Candida*, instillation of Nystatin or Amphotericin B solution into the bladder is indicated. If renal involvement is severe or associated with systemic candidal disease, intravenous Amphotericin B should be used.

### CRYPTOCOCCOSIS (TORULOSIS)

Cryptococcosis or torulosis is a subacute or chronic infection, caused by *Cryptococcus neoformans*, which may involve the lungs, skin or other parts of the body but it has a marked predilection for the brain and meninges (Conant *et al.*, 1954). This pathogenic yeast resides in the soil as a saprophyte.

Although cryptococcosis occurs in all parts of the world, the disease is surprisingly uncommon considering the ubiquity of the causative organism. *Cryptococcus neoformans* has been found in fruit juices, in milk, soil, in pigeon droppings, in various animals and in healthy individuals. However, there is no evidence that transmission of the disease occurs from man to man or from animals to man. It is likely that *C. neoformans* enters the body through the respiratory tract and that even the most common form of cryptococcal infection affecting the meninges is preceded by lung involvement, however mild. Inhalation of cryptococcus containing material from the soil may produce a relatively mild self-limiting influenza-like illness in susceptible individuals.

*Cryptococcus neoformans* is a true yeast and produces a mucoid capsule which is clearly demarcated and whose antigenic biochemical properties have received a great deal of attention recently. Purified extracts have been used in precipitin and agglutination tests and it has been claimed that the capsule is concerned with type specificity.

Hodgkin's disease, leukæmia, sarcoidosis, tuberculosis and systemic steroid therapy predispose to infection with *C. neoformans*. It has been suggested that in experimental infection in mice there is a less marked tissue response in the central nervous system than in other organs and that this may be related to the high incidence of infection of this organ (Levine, Zimmerman & Scorza, 1957; Louria, Kaminski & Finkel, 1963).

### Clinical and Laboratory Features

The disease is commoner in males than in females and, although it may occur at any age, it is seen mainly between the ages of 20 and 60 years. The central nervous system, the lungs, the skin or bones may be involved. Cryptococcal meningitis is the most frequent clinical picture, and strongly resembles tuberculous meningitis in its presentation and behaviour. The

cerebrospinal fluid findings are also similar but the centrifuged fluid contains the yeast-like structures with their capsules and culture on Sabouraud's medium will grow colonies consisting of encapsulated yeast-like cells. Animal pathogenicity tests are indicated in order to make certain that the isolated organism is not a non-virulent variant which also exists. Cryptococcal meningoencephalitis may take the form of an acute infection which may be fatal in weeks or months. Cryptococcal central nervous system involvement may also present as a localized granuloma whose clinical manifestations are those of a space-occupying lesion.

The prognosis of cryptococcal meningitis is bad and death usually results within a year or two of onset of the disease. More chronic cases are also seen in the elderly characterized by progressive mental deterioration.

Pulmonary cryptococcosis may be asymptomatic or may be associated with fever, cough, expectoration, hæmoptysis and chest pain. X-rays may show diffuse lung infiltration, a well defined solitary shadow without hilar enlargement resembling a new growth or lung abscess and a miliary type of shadowing which is indistinguishable from widespread mottling due to other diseases.

Cutaneous lesions occur in about one-sixth of cases of disseminated cryptococcosis. Multiple discrete lesions may also be seen in bones.

### Treatment

A great variety of agents have been tried unsuccessfully in the treatment of cryptococcal meningitis and disseminated cryptococcosis. Assessment of the value of the various preparations is difficult, particularly because of the occasional spontaneous remissions which occur in the chronic form of the disease and this increases the need for prolonged observation when new forms of therapy are evaluated.

Amphotericin B is now established as the only available drug which cures patients with this disease, if the diagnosis is made early. However, following intravenous administration, only very low, hardly discernible levels of this antibiotic are found in the cerebrospinal fluid. If the cerebrospinal fluid contains viable cryptococci despite prolonged therapy with Amphotericin B, intrathecal as well as intravenous administration of the drug is indicated. Littman (1959, 1962) advises intrathecal injections not exceeding 0.7 mg per single dose for an adult administered on alternate days concomitant with intravenous administration, until the spinal fluid is sterile. This may have to be continued for months, until three successive cultures of spinal fluid remain negative for *C. neoformans*. An overdose of intrathecal Amphotericin B may cause hyperpyrexia, arachnoiditis, weakness of the lower extremities and flaccid paraplegia. These complications are reversible, when the drug is discontinued.

Littman has also stressed the importance of thiamine for the capsule synthesis of *C. neoformans*. A thiamine low diet should be given to comatose or semicomatose patients and injections of thiamine or thiamine-rich vitamin B complex should be avoided.

Occasionally, carefully selected patients with a well localized pulmonary lesion may be suitable for surgical excision. This procedure should be preceded and followed by Amphotericin B therapy.

## ASPERGILLOSIS

In this country bronchopulmonary aspergillosis is the most common and most important fungus disease affecting the lungs. Although there are no definite figures giving the incidence of aspergillosis, the size of the problem may be gauged from the fact that Campbell & Clayton (1964) studied 272 patients who attended the Brompton Hospital in London between 1957 and 1962 and who were investigated for bronchopulmonary aspergillosis. *Aspergillus* species may be found either as saprophytes or as pathogens on various parts of the body, particularly in the external ear and the nasal sinuses. Lung involvement and the rare disseminated form are, however, of prime and increasing importance and of world-wide distribution.

Fungi of the genus *Aspergillus* are ubiquitous saprophytes in nature. They are filamentous fungi producing airborne spores which are found throughout the year. Noble & Clayton (1963) have recently shown that, in London, there is approximately a hundred-fold increase in the air count of *Aspergillus fumigatus* spores between the months of October and February. Inhalation of even large numbers of spores, as may occur when compost or mouldy hay is disturbed, usually results at the most in temporary acute tracheobronchitis (Riddell, 1963). However, *Aspergillus fumigatus* may invade and colonize pulmonary tissue in the presence of underlying lung disease. Human aspergillosis is generally due to *Aspergillus fumigatus*, although *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus nidulans* may be responsible. Since these organisms are also common and troublesome contaminants in the laboratory, mere culture of aspergilli is insufficient evidence for the disease and a diagnosis should be based on repeated recovery of the fungus from a suspected lesion and should be supported by clinical, radiological, immunological and, where possible, histological confirmation.

Aspergillosis may rarely occur without any underlying disease. However, it usually affects patients debilitated by disease or who suffer from leukæmia and reticuloses or those under treatment with corticosteroids, cytotoxic drugs and antibiotics.

### Types of Aspergillosis and their Clinical Features

One form of bronchopulmonary aspergillosis is due to saprophytic colonization of lung tissue previously damaged by disease. A well circumscribed mass or network composed of fungal filaments develops and is called an aspergilloma. This ball of fungus is usually within a cavity and occurs almost invariably in the upper lobes of the lung (Fig. 8.4). The cavities in which aspergillomas develop may be caused by tuberculosis, unresolved pneumonia, bronchiectasis, lung abscess, sarcoidosis, pulmonary infarction, pulmonary neoplasia, pneumoconiosis and histoplasmosis.

In extensive *A. fumigatus* infections of a pre-existing apical pulmonary cavity, tension cyst or pleural space, there may be remittent fever, loss of weight and deterioration of health (Riddell, 1963). Yellow-green sputum or empyema pus is produced and this contains masses of fungus mycelium and often sporing structures. Hæmoptysis may occur. Radiologically, a dense tumour-like opacity with a crescentic shadow of air between the intracavitary



inclusion and the wall of the cavity is seen. Tomography is helpful. The contents of the cavity may be observed to move on X-ray screening.

The second form of bronchopulmonary aspergillosis is due to allergy of the patient to the fungus. This allergic type is characterized by the development of transient pulmonary infiltration roentgenologically (Fig. 8.5) and these are associated with pyrexia, wheezing and eosinophilia. Not all bronchial infections by aspergilli are associated with asthmatic manifestations. The culture of *A. fumigatus* from a sputum "plug" or from sputum produced at the height of an allergic episode in a patient with pulmonary infiltration is very strong evidence for the disease. Immediate skin hypersensitivity to prick testing with *A. fumigatus* extracts is present in patients with allergic aspergillosis. Skin hypersensitivity is only found in a patient with an aspergilloma when coexistent allergic hypersensitivity exists. Serum precipitins are an almost invariable finding in patients with aspergillomata but are found in only about 70 per cent of allergic aspergillosis patients (Campbell & Clayton, 1964).

Finally, the third type of infection due to *A. fumigatus* is disseminated aspergillosis. This form is predisposed by severe underlying disease, e.g. carcinoma, leukæmia, Hodgkin's disease, or steroid and antibiotic therapy. Spread to other organs, including the brain, meninges, kidney, heart, lymph nodes, skin, etc., has been recorded. The clinical manifestations are related to the organs involved.

### Treatment

There is some argument as to whether *A. fumigatus* is merely a secondary invader of previously diseased lung tissue or whether it is a pathogen in its own right. This has a bearing on the prognosis and therapy of aspergilloma. The prognosis in cavitory and space infection is favourable, spontaneous resolution may occur (Riddell, 1963) and consequently once a confident diagnosis of aspergilloma has been made, no specific treatment is indicated apart from the appropriate therapy of the underlying lung disease. Plummer (1960) subscribes to the view that spontaneous remission of saprophytic pulmonary aspergillosis follows successful treatment of the primary lung disease, but Edwards and La Touche (1964) disagree and include three cases of mycetoma and four of saprophytic aspergillus bronchitis in their series of patients treated with the tetræne antifungal antibiotic pimarin.

However, in severe infections of the bronchial tree, oral Nystatin and inhalations of Nystatin, brilliant green and hydroxystilbamidine have been found of value. In very severe bronchopulmonary infection and in the disseminating form, intravenous Amphotericin B is used. Surgical resection of an aspergilloma may be successful, although bronchial fistula or empyema may develop.

The allergic form of aspergillosis may continue for many years and be punctuated by episodes of wheezing, raised temperature, eosinophilia and consolidation-collapse on X-ray. Some of these patients may recover spontaneously, others get gradually worse and may go into status asthmaticus. Riddell suggests inhalations of aerosols containing Nystatin, brilliant green or 2-hydroxystilbamidine, and attempts at desensitization with *Aspergillus* antigen and corticosteroids in certain cases. Edwards & La Touche (1964)

have claimed benefit from pimaricin given as an aerosol 2–3 times daily as a 2·5 per cent suspension diluted in an alkaline agent in doses of 2·5 mg. The polypeptide antibiotic X-5079 C has also been reported to be of value.

### COCCIDIOIDOMYCOSIS

This very common endemic infection is caused by *Coccidioides immitis*, a filamentous organism which segments into spores and which has been repeatedly isolated from soil. Winds and air-currents disseminate the infected soil and the inhaled dust causes a transient influenza-like infection. The inhaled spores, which are about  $2\mu \times 5\mu$  large, change in the lung or in other tissues into thick walled spherical structures known as sporangia or spherules which are 20–60 $\mu$  in diameter and become filled with small endospores which are released following rupture of the wall of the spherule and in turn develop into sporangia.

The disease is confined to North and South America, particularly the arid South-western parts of the United States, Central America and Mexico. It is very common in the San Joaquin Valley of California and coccidioidomycosis is sometimes called Valley Fever. All ages and both sexes are affected but the progressive form of the disease is much commoner in males and affects more often and more severely darker skinned races, i.e. Negroes, Mexicans, Indians, etc.

#### Clinical Features

The disease is represented by two completely separate forms—primary and progressive coccidioidomycosis.

*The primary infection* takes place 10–18 days after exposure to *C. immitis* and is asymptomatic in about 60 per cent of those infected. In the remainder, primary coccidioidomycosis is characterized by a brief respiratory infection with low grade fever, cough, headache, pain in the chest, joint pains and sore throat. Occasionally, expectoration and hemoptysis may occur. One to three weeks later erythema multiforme or erythema nodosum may appear. Signs of bronchitis or pleurisy may be found; there may be a raised sedimentation rate and leucocytosis. The coccidioidin skin test becomes positive, X-ray changes include scattered shadowing, mainly hilar or more diffuse consolidation, occasionally hilar adenopathy or pleural effusions.

The great majority of primary pulmonary coccidioidomycosis infections clear up completely, but cavitation may persist in a very small number of infected persons. A persistent granulomatous lesion may also occur.

*The progressive infection* may be the immediate outcome of the primary disease or it may develop even 5–6 months later.

Only about 1 in a 1,000 cases of primary coccidioidomycosis becomes progressive. Clinically, there is gradual loss of weight, fever, loss of appetite and signs of pulmonary disease. Dissemination may occur to other organs or to a single organ. In the skin, simple or multiple subcutaneous cold abscesses may develop; the bones (Fig. 8.6), the meninges, the brain or lymph nodes may be involved in a granulomatous reaction. A solitary granuloma of the skin may be seen occasionally. The primary infection may fail to resolve completely and symptomless cavitation in the lung may be discovered radiologically. This may persist for years and the cavity may have to be differentiated

from tuberculosis. Persistent nodular shadows may easily be confused with bronchogenic carcinoma. Even in coccidioidomycosis endemic areas and in the presence of positive immunological evidence for the disease, a thoracotomy may be required to settle the diagnosis, if carcinoma is suspected.

X-ray changes may show varying degrees of patchy or confluent infiltration (Fig. 8.7). Hilar adenopathy is not uncommon, miliary shadowing similar to that seen in tuberculosis, but usually with more ill defined lesions, may be found. Thin walled cavities usually without but sometimes with a fluid level (Figs. 8.8 and 8.9) are particularly suggestive of the disease in endemic areas.

The sputum of patients with the progressive form of the disease often contains sporangia and these may also be found during the primary infection. Culture of sputum, or bronchial and stomach aspirates should be carried out. Animal inoculation with infected material is of diagnostic value.

Smith and colleagues (1955) have contributed greatly to the diagnosis and pathogenesis of coccidioidomycosis with their immunological, epidemiological and pathological studies. An extract of the fungus (coccidioidin) injected intradermally produces a positive reaction some four weeks after infection and this conversion is analogous to the Mantoux reaction in tuberculosis. A positive coccidioidin skin test is essentially specific (with the exception of rare cases of cross-sensitivity reaction) and implies present or past infection. Humoral antibodies can be detected after the development of skin sensitivity. They are generally absent in silent primary infections but are found in a high percentage of primary infections with severe clinical manifestations. Precipitins are detectable earlier than complement fixing antibodies but the titre of antibodies rises with the progress and severity of the disease whereas the precipitin titre tends to fall and therefore does not carry the same serious prognostic significance as do the complement fixing antibodies.

### Treatment

The great majority of patients with primary coccidioidomycosis, including those with severe symptoms, recover spontaneously and need no active treatment. Intelligent interpretation of tests showing the development of immunity in patients is of help in assessing the prognosis and progress of the disease. The presence of erythema nodosum, erythema multiforme or a silent pulmonary cavity in the course of the primary infection is usually associated with the development of good immunity. A rising complement fixation titre suggests threatening dissemination. Radiological follow-up of pulmonary lesions is important and helps in deciding the indications for intravenous Amphotericin B therapy. Extending residual pulmonary lesions of the primary type do not break down, need no active treatment and must be differentiated from the disseminated form. There is said to be a 60 per cent mortality in the progressive disease.

Coccidioidal meningitis, if untreated with Amphotericin B, is invariably fatal within 1-2 years. Winn (1962) has summarized the indications for Amphotericin B in coccidioidomycosis:

1. Extending or exacerbating chronic pulmonary lesions.
2. Threatened or actual dissemination.

3. Coccidioidal meningitis.
4. Ancillary to surgical treatment in:
  - (a) Removal of pulmonary residual lesions.
  - (b) Empyema, decortication (cavity rupture).
  - (c) Excision of bone lesions. Fusion of joints.
  - (d) Drainage of peripheral and deep abscesses. Removal of infected lymph nodes, sinus tracts. Epididymectomy, orchiectomy.
5. Prophylaxis against dissemination of disease during pregnancy.

### HISTOPLASMOSIS

This disease is caused by *Histoplasma capsulatum*, a fungus which acts as a parasite of the reticuloendothelial system and which has a typical filamentous phase in culture, but in tissues appears as a small yeast-like intracellular body 1–5  $\mu$  in diameter. The organism belongs to the group of opportunistic fungi in that although it is normally found in the soil of endemic regions as a saprophyte, its spores may be inhaled in dust and cause disease.

Histoplasmin testing is a most useful epidemiological tool for defining the geographical distribution and prevalence of infection with *H. capsulatum*, although cross-sensitivity reactions with coccidioidin and blastomycin detract from its specificity (Edwards, 1958). In addition to its high incidence in America, endemic areas with a low incidence exist in Africa and Asia and some two dozen cases of histoplasmosis have been reported in Great Britain during the past few years. All these patients had been in endemic areas previously and it seems that *H. capsulatum* is not endemic in Europe. Murray & Sladden (1965) described a case of disseminated histoplasmosis following long-term steroid therapy for reticulosarcoma in a Scotswoman who had lived for many years in India and returned home to Great Britain in 1952. They thought that primary infection probably occurred in India and reactivation of the infection took place in presence of reticulosis and steroid therapy.

Histoplasmosis, like coccidioidomycosis, is clinically characterized by a primary benign respiratory infection and a progressive serious disseminating phase. Its clinical and radiological features also resemble tuberculosis from which it may be difficult to differentiate and with which it may rarely coexist. In children, the portal of entry is probably the gastrointestinal system, the abdominal structures are predominantly hit by the disease and the lung involvement is likely to be a pre-terminal phenomenon due to dissemination.

### Clinical and Radiological Features

All ages are susceptible but children are more liable to develop the progressive form of the disease.

Primary histoplasmosis is generally a benign pulmonary infection, in the large majority of patients asymptomatic, recognized retrospectively by an association of diffuse, dense, regular, round opacities and hilar gland enlargement, calcified in the later stages on X-ray and a positive histoplasmin reaction. In the more chronic form of the primary lung infection, there may be symptoms and signs of bronchitis or pneumonia or the clinical picture may resemble chronic pulmonary tuberculosis. There may be lassitude, fever,

dyspnoea, night sweats, chest pain and loss of weight extending over a period of several months. At this stage, the chest X-ray may show disseminated pneumonic foci with or without hilar gland enlargement. Some of these infiltrates may resolve, others undergo fibrosis and gradually calcified nodules, which may be surrounded by a paler halo, appear. These are characteristic of histoplasmosis. This miliary calcification is not easily distinguished from the calcified foci of tuberculosis, but in histoplasmosis there is less evidence of aggregation, the calcification is more dense and evenly scattered over the lung fields (Fig. 8.10) without the predominantly upper zone distribution of calcified foci seen in tuberculosis (Pierce, 1958).

Progressive histoplasmosis may occur at any age, but is more common in very young children, in old age and in debilitated individuals. In adults, extension of the disease due to reinfection may occur, but the primary lesion may be extrapulmonary and muco-cutaneous granulomatous lesions around the mouth are common. Loss of weight, fever, anaemia, leucopenia and hepatosplenomegaly are characteristically present. In children, involvement of the reticuloendothelial system is even more marked and the disease clinically resembles miliary tuberculosis. In the more chronic progressive form, the disease both clinically and radiologically resembles fibro-caseous tuberculosis, producing progressive fibrosis and cavitation (Pierce, 1958).

Approximately 0.1 per cent of cases of histoplasmosis go on to the progressive disease which is fatal in a large majority of the patients with dissemination and in about one-third of the patients with the chronic pulmonary form of the disease.

### Treatment

In a study sponsored by the United States Public Health Services, Lynch, Furcolow & Doto (1962) have evaluated the indications and results with intravenous Amphotericin B in this disease. They suggest that in severe acute pulmonary histoplasmosis, which is only rarely fatal but which is often associated with a severe protracted clinical illness, Amphotericin B given for 7-14 days gives prompt relief of symptoms and improves X-ray appearances and its administration is therefore justified. In the acute disseminated form which is most commonly seen in children and in old age and which has a mortality rate of 80 per cent, the drug is life-saving. Finally, in disseminated disease of the chronic progressive cavitory type, the drug has significantly improved the clinical and X-ray pictures.

### NORTH AMERICAN BLASTOMYCOSIS

This granulomatous infection is caused by *Blastomyces dermatitidis*, a dimorphic fungus which is seen in tissues in the yeast phase carrying a single bud. It is probable that the infection is acquired from soil through the respiratory tract. It is endemic in some south eastern regions of the United States and it affects mainly males.

The disease may be encountered as a pulmonary infection or in the disseminated form. The latter is the more common and may affect the skin, bones, the genito-urinary system, particularly the prostate and the central nervous system. The skin lesions which are part of the disseminated disease are usually well defined verrucous granulomata which spread peripherally.

The diagnosis is confirmed histologically by finding the yeast-like fungus in the tissues. The blastomycin test is positive but may remain negative in severe disseminated infections. Rarely primary inoculation of the skin may produce a chancriform lesion with lymphangitis and lymphadenitis without generalized spread.

Occasional pulmonary involvement only may be met, but this is more usually part of the systemic disease. Cough, dyspnoea, fever, chest pain and pleurisy may occur and both symptomatology and radiological findings of bronchopneumonic shadowing, hilar gland enlargement or occasional miliary shadowing resembles the changes seen in tuberculosis or pulmonary actinomycosis.

Intravenous Amphotericin B is the treatment of choice.

### **SOUTH AMERICAN BLASTOMYCOSIS (Paracoccidioidomycosis)**

This is caused by *Blastomyces (Paracoccidioides) brasiliensis*. The large thick walled yeast cell is found in tissues and has characteristically multiple buds at its periphery. The disease is seen exclusively in South America. Multiple granulomatous lesions, spreading along lymphatic channels, are seen mainly on the skin. These verrucous lesions may affect the mucocutaneous surfaces and are disfiguring and destructive. Systemic spread to viscera is seen and in the lungs resembles miliary tuberculosis, the other disseminating fungus diseases or the malignant lymphomas.

Sulphadiazine has a suppressive effect. Amphotericin B is curative.

### **SPOROTRICHOSIS**

Sporotrichosis is a chronic subcutaneous fungus infection caused by *Sporotrichum schenkii*, a diphasic aerobic fungus which is introduced into the skin in its mycelial form as a result of trauma and which proliferates in the tissues or in pus in the form of oval yeast cells. A subcutaneous nodule develops which may eventually ulcerate through the skin. The infection spreads along lymphatic channels proximally and other subcutaneous granulomata with overlying superficial ulceration and crust formation result (Fig. 8.11). The disease has a world-wide distribution, is commoner in males and affects mostly farmers and horticulturists who come into contact with the organisms which thrive on various woods, plants and soil. It enters the skin through abrasions and the lesions are most commonly seen on the hand, arm, foot or leg. Some days or weeks after introduction of the pathogen into the skin, a painless pustule enlarges to break down into an ulcer with an undermined edge. Several weeks later, a chain of nodules or ulcers appears along the course of the lymphatics and persists.

Disseminated sporotrichosis is rare and may be due to spread of infection from the primary site in the skin in patients with a low degree of immunity but it is more likely that dissemination occurs in those who have inhaled or ingested the organism. The lungs, bones, viscera or central nervous system may be affected.

The intradermal test with sporotrichin is positive with present or past

infection, but may be negative in the disseminated disease. Precipitation and complement fixation tests are of doubtful value.

The cutaneous form of the disease responds well to treatment with potassium iodide. In patients who do not tolerate iodides or in resistant cases, local injections of Amphotericin B or X-5079 C have been claimed to be successful. Although iodides are also effective in the systemic disease, intravenous Amphotericin B is the drug of choice in this. Oral griseofulvin has been claimed to be useful, but the reports about this are conflicting.

### Acknowledgements

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## CHAPTER 9

# HYPERTENSION

by

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### Introduction

DURING the past decade a great expansion has occurred in knowledge and understanding of the functions of the renal enzyme renin, and its product angiotensin. Since it would be scarcely practicable to discuss recent advances in clinical hypertension without reference to renin, the first part of this chapter will be devoted to a brief survey of current views of the role of renin and angiotensin in health and disease.

This section will necessarily deal with many aspects in which hypertension does not feature. Moreover, it is expressly didactic, and contains a minimum of references. For a more extensive bibliography more detailed reviews should be consulted (Braun-Menendez, 1956; Page & Bumpus, 1961; Haas & Goldblatt, 1963; Peart, 1965; Helmer, 1965; Brown *et al.*, 1966, 1967).

The second part of the chapter will be concerned with general clinical problems of current interest in which hypertension occurs: renal artery stenosis, aldosterone-secreting tumours, the differential diagnosis of hypertension with hypokalaemia, and phaeochromocytoma.

### RENIN AND ANGIOTENSIN

#### Chemistry

Renin is an enzyme which is present in extracts of the renal cortex and which acts on a substrate, angiotensinogen, present in plasma, to form the decapeptide, angiotensin I (Fig. 9.1). Both renin and angiotensin I are largely, if not entirely, devoid of direct pharmacological effects. The active octa-

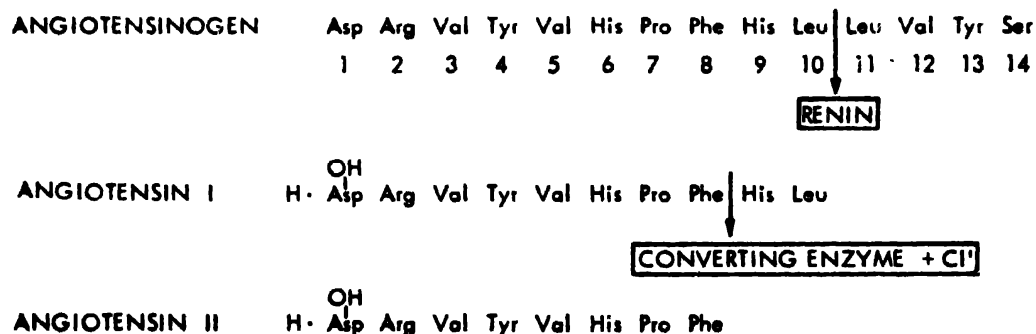


FIG. 9.1. Diagram of aminoacid sequence in angiotensinogen (renin-substrate), angiotensin I, and angiotensin II; with site of action of renin and "converting enzyme".

peptide angiotensin II is formed on removal of the two C-terminal amino-acids by a converting enzyme in plasma (Fig. 9.1). Angiotensin II is, in turn, broken down into smaller inactive fragments by various peptidases present in blood and other tissues, and known collectively as angiotensinase.

In the mammalian kidney, renin is closely associated with the vascular pole of the glomerulus, although it is not yet certain whether the macula densa, a plaque of specialized cells in the wall of the distal renal tubule, or the juxta-glomerular granular cells in the media of the afferent glomerular arteriole, is the principal storage site. Normally the glomeruli nearer the surface of the kidney are richer in renin than those adjacent to the medulla.

Although the presence of renin in blood was for long disputed, it is now known that small quantities circulate in peripheral venous and arterial plasma. The concentration of renin in renal venous plasma has been found to be slightly but significantly higher than that in the renal artery or peripheral veins, suggesting that the kidney secretes renin into renal venous blood. Renin-like enzymes have also been demonstrated in high concentration in renal lymph and in urine. There are therefore three major routes by which renin leaves the kidney, and all of these must be considered when estimating the renin secretion rate.

Renin-like enzymes have also been demonstrated in the arterial wall, in salivary glands, and in the uterus, placenta and amniotic fluid.

Angiotensin is less easy to detect and to identify than renin but has been found in extracts of whole blood, plasma, renal lymph and possibly amniotic fluid.

Many different methods are at present employed for the estimation of renin and angiotensin, and these vary widely in accuracy, specificity, and in the terms in which the results are expressed. This may make direct comparison of results obtained in different laboratories difficult. At present, three main types of assay are likely to be encountered:

*Angiotensin concentration:* this presents no difficulties of expression, since synthetic angiotensin is commercially available, and the angiotensin content of tissue extracts may be compared with this standard, and determined in units of weight. However, most of the methods so far available for the estimation of angiotensin in extracts of blood or plasma fail to detect it in a proportion of normal subjects. Such techniques are therefore restricted to demonstrating levels above the normal range.

*Renin concentration:* the enzyme is extracted, freed from substrate, angiotensinases, activators and inhibitors, and then incubated with a standard preparation of substrate under controlled conditions. The velocity of angiotensin formation in the incubation mixture is in these circumstances directly proportional to the concentration of renin in the extract.

Results are expressed in relation to a standard preparation of renin of the appropriate species. At present there is no international renin standard and results are reported in terms of enzyme units peculiar to each laboratory. However, direct comparison of the enzyme units of different laboratories may be made by exchanging standard renin and substrate preparations.

*Renin activity:* this term is applied generically to a group of methods in which renin and substrate are usually extracted together from a plasma sample, and angiotensinases are either destroyed or inhibited. The extract

is then incubated, and the amount of angiotensin formed after a measured interval (often 3 hours) is determined. The rate of angiotensin formation depends on both the renin concentration and substrate concentration in the extract; with several of these methods evidence of activators or inhibitors has also been found. The results are not therefore expressed in enzyme units, but simply as the quantity of angiotensin formed per unit of time. As is the case with angiotensin assays, many of these techniques fail to detect renin activity in a proportion of normal subjects.

The different methods employed for the estimation of renin, angiotensin and renin activity makes comparison of results difficult, but in general there is rough agreement between the values obtained. It is of course important when interpreting results obtained with any given method to know the normal range for that technique.

### Actions

As far as is known, the various actions of renin are mediated by angiotensin. Many of the main effects, which are summarized below, are closely inter-dependent.

*Pressor Action.* The systemic pressor effect of injected renin was first noted by Tigerstedt & Bergman in 1898, and this continued to attract most attention until recently. The experiments of Goldblatt and his colleagues (1934), which showed clearly that hypertension could be produced experimentally by renal artery constriction, led to the suggestion that renal ischæmia might cause the release of increased amounts of renin into the circulation, and then, via angiotensin, produce hypertension by a direct vasoconstrictor action. Further work has shown that this hypothesis is not tenable in a simple form, although a consideration of the relationship between sodium balance and the pressor sensitivity to renin and angiotensin, which is discussed more fully in a later paragraph, suggests that it may be valid with substantial modifications.

Angiotensin has also been observed to increase the pulmonary artery pressure, and to cause vasoconstriction in the splanchnic circulation (De Bono *et al.*, 1963).

*Effect on Adrenal Cortex.* Recent knowledge of this aspect of the renin-angiotensin system has resulted from several widely separated lines of investigation.

Deane & Masson (1951) found that the administration of renin to rats led to histological changes in the adrenal cortex suggestive of increased activity. Sevy & Wakerlin (1953) tentatively suggested that renin might stimulate the pituitary-adrenal system to increase the secretion of mineralocorticoids and hence cause a compensatory decrease in renin release.

Hartroft & Hartroft (1953) and Tobian (1960a, b) showed that sodium deprivation caused histological changes in the juxtaglomerular apparatus which suggested increased secretory function, while the converse occurred during sodium loading.

These and other observations led Gross (1958, 1960) to postulate that renin and angiotensin might serve to control sodium excretion by regulating aldosterone secretion. Subsequently, Genest and co-worker (1960) and

Biron and co-worker (1961) showed that angiotensin caused an increase in aldosterone excretion in man, and several studies have since demonstrated an increase in the aldosterone secretion rate after the administration of angiotensin or renin in other species.

Whether or not angiotensin stimulates the secretion of other corticosteroids under normal physiological conditions is disputed (Davis, 1963; Slater *et al.*, 1963, 1965), although there is little doubt that large (and possibly non-physiological) doses are capable of such effects. A sustained rise in the peripheral venous plasma concentration of aldosterone, without a corresponding change in plasma cortisol or corticosterone, occurs during intravenous infusions of angiotensin in normal subjects (Fraser *et al.*, 1965).

*Effect on Adrenal Medulla.* The release of adrenaline after the administration of angiotensin to dogs and cats has been reported (Braun-Menendez *et al.*, 1940b; Kaneko, McCubbin & Page, 1961; Feldberg & Lewis, 1965).

*Release of Kinins.* There is good, but at present inconclusive, evidence that angiotensin may similarly stimulate the release of kinins into the circulation (Robertson, Peart & Andrews, 1962; Brown *et al.*, 1966).

*Actions on the Nervous System.* Lewis & Reit (1965) demonstrated that angiotensin stimulates autonomic ganglia, and Lavery (1963), Benelli, Della-Bella & Gandini (1964), Yu & Dickinson (1965) and Scroop, Walsh & Whelan (1965) have also suggested that some of the effects of angiotensin are mediated by the nervous system.

*Internal Distribution of Electrolytes.* Angiotensin influences the distribution of electrolytes within the body, promoting the movement of sodium into, and potassium out of, vascular smooth muscle cells (Friedman & Friedman, 1964, 1966).

*Diuretic and Antidiuretic Actions.* Infusions of renin or angiotensin frequently cause proteinuria, and have marked, but very varied, effects on the urinary excretion of electrolytes and water. Usually, natriuresis and antinatriuresis accompany corresponding changes in water excretion, the particular nature of the response in an individual instance depending on the dose given, the duration of administration, the height of the arterial pressure before infusion, the state of sodium balance, and the prevailing corticosteroid level. For various reasons it is unlikely that these diuretic or antidiuretic effects are a simple consequence of angiotensin-stimulated increases in aldosterone, or of acute changes in renal artery pressure.

*Possible Intrarenal Roles of Renin and Angiotensin.* It is possible that renin has several intrarenal functions, such as the regulation of glomerular filtration rate, renal blood flow, renal medullary circulation and sodium reabsorption by the renal tubule. Such local actions might be caused by renin or angiotensin carried in renal lymph, and could be effected without altering the blood levels of renin sufficiently to influence more distant target organs. This suggestion carries the further implication that the systemic actions of renin and angiotensin may have evolved as secondary and supplementary to the intrarenal actions.

At present the precise nature (if any) of such local intrarenal effects is undecided, largely because of the absence of sufficiently sensitive assay techniques to detect changes in renin in single nephrons.

### Relationship between Renin, Aldosterone and Sodium Balance

Increases in the concentration of renin in peripheral blood have been demonstrated in a variety of normal and pathological situations in which aldosterone production is elevated. Circulating renin is increased on tilting from the recumbent to the upright posture, and is generally higher during

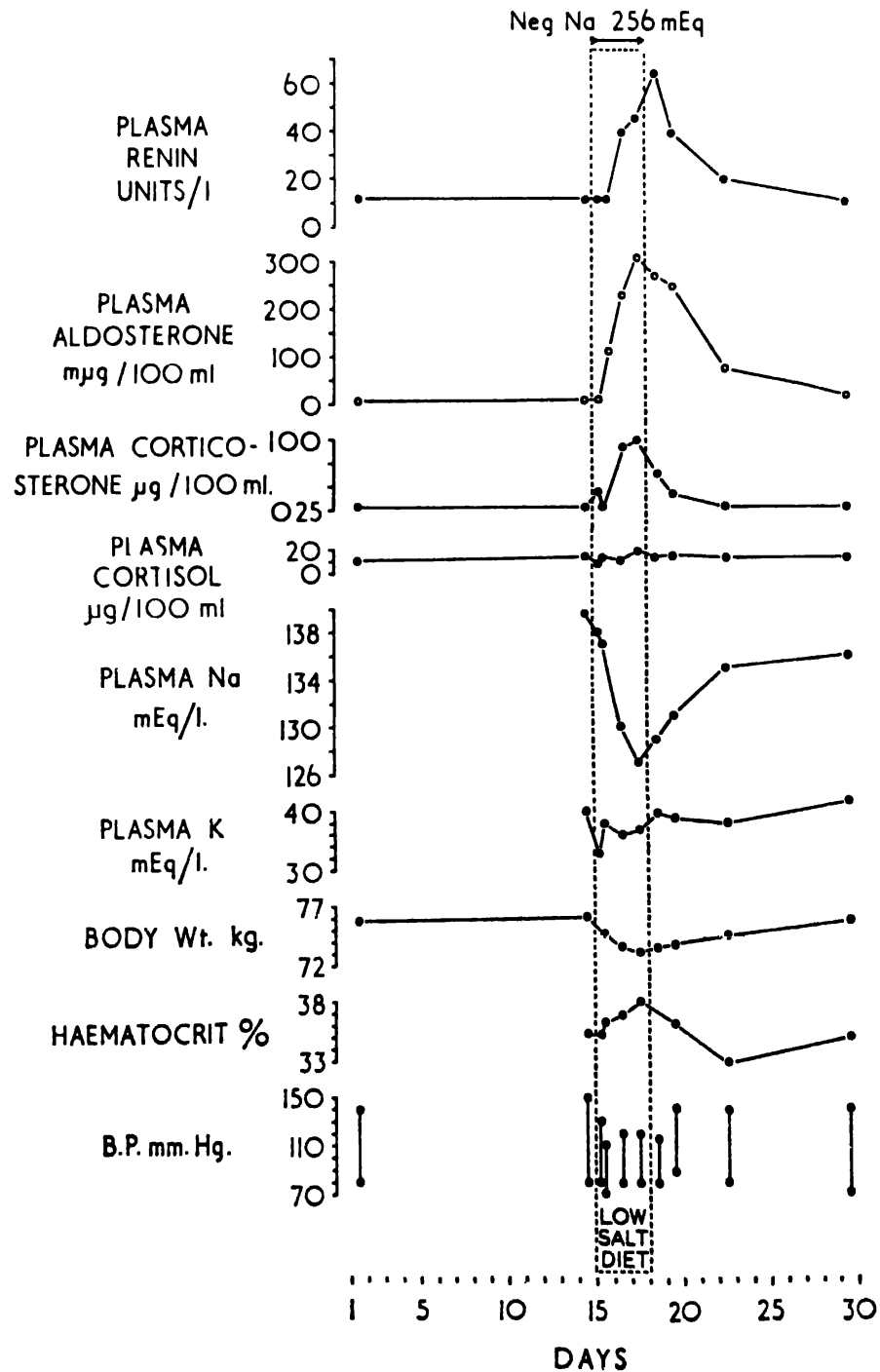


FIG. 9.2. Changes in renin, plasma steroids, plasma sodium, plasma potassium, weight and haematocrit during experimental sodium restriction in a patient with sodium-losing renal disease. A net loss of 256 mEq.Na occurred whilst on a low-salt diet. (Brown *et al.*).

the day than at night. There is a slight increase during the luteal phase of the menstrual cycle, and during normal pregnancy renin is often, although not always, increased to well over the non-pregnant range.

In normal subjects, renin is increased by dietary sodium restriction or by natriuresis, and decreased by sodium loading. In patients with renal disease and defective ability to conserve sodium, the extent of the sodium loss, and hence of the rise in renin, is very much greater (Fig. 9.2). Renin has also been observed to be increased following a large hæmorrhage, in some cases of the nephrotic syndrome, and in hepatic cirrhosis with ascites. Results in cardiac failure are variable; some patients have a plasma renin concentration which is normal before treatment and rises after diuretics have been administered, whereas other patients have abnormally high levels of circulating renin before therapy, the renin then falling to normal with the relief of œdema. It is noteworthy that the aldosterone secretion rate is also very variable in cardiac failure. These findings all support the concept that renin, via angiotensin, regulates the secretion of aldosterone in response to changes in sodium balance.

In two situations renin and aldosterone are notably divergent. In Addison's disease, where aldosterone production is defective, renin is abnormally high, falling as the electrolyte abnormalities are corrected with steroid therapy. Conversely, when an excess of aldosterone is produced by an adrenocortical adenoma, the total exchangeable sodium is abnormally high and renin abnormally low. These findings are entirely consistent with renin being concerned with the regulation of aldosterone, and indicate that the state of sodium balance governs the circulating renin level and thus the appropriateness of the adrenocortical stimulus in particular circumstances.

#### **Possible Antidiuretic Role of Renin and Angiotensin in Diabetes Insipidus and Other diseases**

The polyuria of diabetes insipidus can be reduced by the administration of various diuretics (Meyer, 1905; Bauer & Ashner, 1924; Crawford & Kennedy, 1959; Kennedy & Crawford, 1959; Havard & Wood, 1961; Blom, Rook & Sonneveld, 1963). This effect is probably related in some way to the change in sodium balance, since it can also be obtained by dietary sodium restriction (Fitz, 1914; Beaser, 1947), while the drug-induced anti-diuresis is preceded by an increased sodium excretion and can be aborted by salt replacement (Havard & Wood, 1961). The phenomenon may therefore be due, in part at least, to the action of renin and angiotensin (Brown, Lever & Robertson, 1965d), since both sodium restriction and diuretic therapy provoke an increase in plasma renin concentration.

It is of interest that this form of therapy may be effective in both vasopressin-sensitive and vasopressin-resistant diabetes insipidus. If angiotensin is important in this phenomenon, and if, as Lever (1965) has suggested, the renal effects of vasopressin and angiotensin are primarily vascular, a different response of the intrarenal vessels to vasopressin and angiotensin is implied in nephrogenic diabetes insipidus. It is known that angiotensin and vasopressin have widely differing actions from each other at various parts of the vascular bed.

Defective ability to excrete a water load is found in several conditions in

which the plasma renin concentration is increased. These include Addison's disease, hepatic cirrhosis, and anorexia nervosa. This raises the possibility that in these diseases also, the abnormality of renal function may, at least partly, be due to increases in renin and/or angiotensin, either in blood or within the kidney.

### **Inverse Relationship Between Plasma Sodium and Renin Concentration**

In many situations, sodium deficiency is accompanied by a fall in plasma sodium concentration, and it is therefore not surprising that in several conditions, plasma renin concentration is inversely related to plasma sodium. This has been observed in individual patients with Addison's disease and sodium-wasting renal disease (Fig. 9.2) and in a large series of patients with

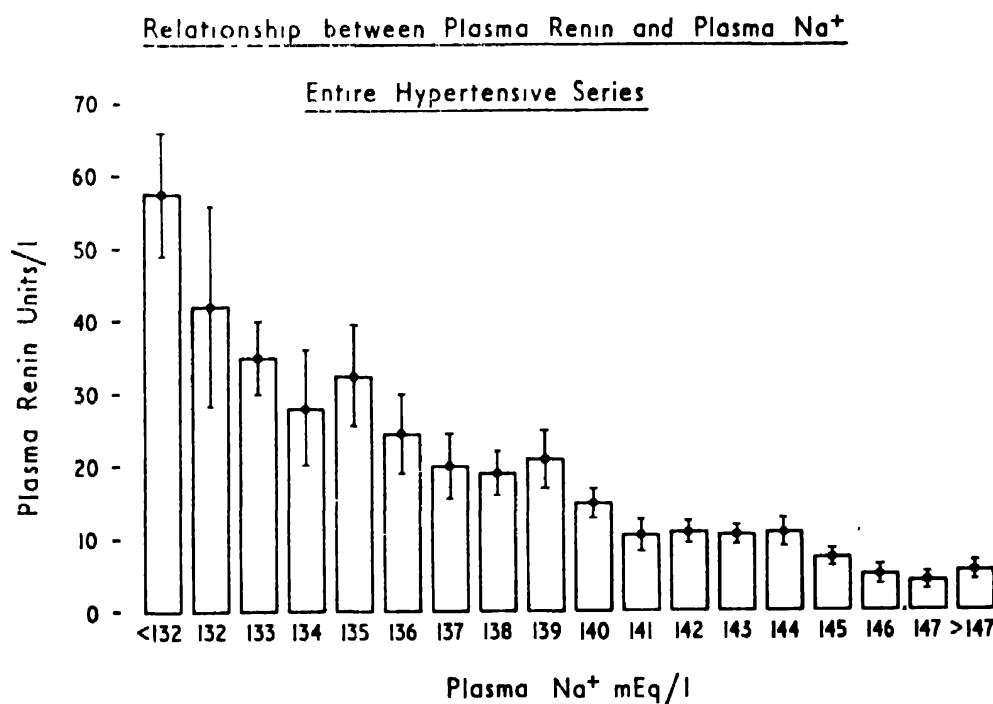


FIG. 9.3. Inverse relationship between plasma sodium and renin concentration in hypertension. Mean and S.E.M. shown for renin concentration. Figure based on 399 pairs of observations in 253 patients with hypertension of varied aetiology. Treated and untreated cases are included.

cardiac failure, including both treated and untreated cases. The relationship has also been found in hypertension (Fig. 9.3) and is apparently independent of aetiology, severity, complications and treatment.

Plasma sodium and plasma renin are not invariably related in this way, however; where very low plasma sodium concentrations are not a reflection of sodium depletion, as in patients with an excess of antidiuretic hormone, plasma renin concentration may be normal, or even abnormally low.

### **Relationship between Plasma Renin Concentration and the Pressor Sensitivity to Renin and Angiotensin**

While changes in sodium balance produce inverse changes in plasma renin concentration, the pressor sensitivity to renin and angiotensin is also affected. Several studies have shown that sodium loss diminishes, and sodium

loading increases, the pressor effect of infused or injected renin and angiotensin. This implies therefore that the amount of renin or angiotensin required to produce a given rise in arterial pressure is directly related to the prevailing plasma concentrations of these substances. Thus where renin is reduced, as in patients with primary hyperaldosteronism and normal subjects loaded with sodium, increased sensitivity to the pressor effect of angiotensin is found.

Conversely, the pressor effects of angiotensin are reduced in several conditions—pregnancy, cirrhotic ascites, sodium deprivation, Addison's disease, and the syndromes reported by Bartter and co-workers (1962) and Desmit (1964)—in all of which plasma renin concentration has been shown to be raised. Thus small absolute increments in plasma renin concentration might affect the blood pressure in conditions in which renin is low (such as primary hyperaldosteronism), whereas a large increase in renin would be needed to produce a pressor effect when the circulating renin is high (as in Addison's disease). Kaplan & Silah (1964) similarly suggested that the sensitivity to infused angiotensin might reflect its plasma concentration. It must, however, be emphasized that an explanation of this kind presupposes that the concentration of renin in plasma is within a range capable of affecting blood pressure. This important proviso has recently been tested in experiments in which pressor infusions of renin into the rabbit (Imbs *et al.*, 1967) and of angiotensin into man (Mulrow, 1964) were associated with three-fold to four-fold increases in the plasma concentrations of these substances, changes which are not large in relation to the wide range of plasma level found in different clinical circumstances.

It seems entirely possible on the present evidence that renin and angiotensin may be involved in several ways (including a direct pressor action) in the maintenance of a normal blood pressure and the induction of renal hypertension,

Several lines of research have indicated that sodium is an important factor in the pathogenesis of hypertension, and a number of attempts have been made in recent years to synthesize the available evidence into a uniform concept. These ideas are discussed in more detail elsewhere (Brown *et al.*, 1966; Brown, Lever & Robertson, 1967). It is probable that for reasons already given, one way in which sodium retention may provoke hypertension is by "sensitising" the animal to the pressor effects of angiotensin. Pressor sensitivity thus is probably as important as the absolute level of renin or angiotensin.

### **Renin and Angiotensin in Clinical Hypertension**

A decade or so ago, it was usual to think of renin as responsible for renal hypertension in the sense that a renal lesion in some way released into the circulation an excess of renin, which then caused hypertension by a direct vasoconstrictor action.

More recently two further notions have emerged; that renin is high in the malignant phase of hypertension and that it is abnormally low in patients with an aldosterone-secreting adrenal adenoma. These views have some truth, but neither is entirely accurate without qualification. Renin cannot be considered in hypertension apart from its functions in normal physiology.



Several of these are at present imperfectly understood, but the relationship between renin, sodium balance, and aldosterone secretion has been extensively studied, and provides a basis from which to consider the wide variations in renin which are observed in different forms of clinical hypertension. The general inverse relationship between plasma sodium and renin concentration referred to above may be considered as one aspect of this renal-adrenal system.

### **Hypertensive Syndromes with Low Plasma Renin Concentration**

*Adrenocortical Overactivity.* The simplest example is the adrenal adenoma (Conn's tumour) secreting an excess of aldosterone, and hence leading to an abnormally high total exchangeable sodium, increased pressor sensitivity to infused angiotensin, and low plasma renin concentration. A rather similar situation may occur in some cases of Cushing's syndrome. In primary hyperaldosteronism, the depression of circulating renin can be shown to be due to electrolyte imbalance rather than to the excess aldosterone directly, since treatment with an aldosterone antagonist such as spironolactone promotes sodium diuresis and increases plasma renin concentration to normal or even raised levels, while the adenoma produces undiminished quantities of aldosterone.

*Pre-eclampsia.* Plasma renin is frequently elevated in normal pregnancy, but in the hypertensive disease of pregnancy (pre-eclampsia) the mean plasma renin is significantly lower than at a comparable stage of normal pregnancy; patients with the most severe disease have in general the lowest plasma renin concentrations. In this disease, as in Conn's syndrome, the pressor sensitivity to infused angiotensin is relatively enhanced.

### **Hypertensive Syndromes with Increased Plasma Renin Concentration**

*Sodium Depletion; Diuretics.* Dietary sodium restriction or the administration of natriuretic drugs can produce increases in plasma renin in hypertensive patients as in normal subjects. In the absence of renal disease, usually only moderate increases in renin are produced in this way. In patients with renal failure, however, in whom the renal control of sodium balance is erratic, even without diuretics, large sodium deficits and corresponding increases in renin are readily produced.

*The Hyponatraemic Hypertensive Syndrome.* This interesting condition is the only form of hypertension in which plasma renin concentration has been found consistently raised. It is characterized by low plasma sodium levels, severe hypertension (frequently in the malignant phase), and either stenosis of a main renal artery or parenchymatous renal disease. Where studied, aldosterone excretion, secretion rate and plasma concentration have been elevated, and probably as a consequence, plasma potassium has been low (Brown *et al.*, 1965b). All these distinctive features may disappear following correction of a renal artery stenosis, unilateral nephrectomy, or adequate control of the blood pressure with hypotensive drugs (although this may be difficult to achieve in some instances).

Detailed metabolic studies of cases of the type are rare, and many aspects of the pathogenesis of the syndrome are uncertain. The distinctive hyponatraemia may be due to external loss of sodium, internal transfer of sodium

and water, dilution of plasma sodium following defective water excretion, or a combination of one or more of these. Increased urinary sodium excretion seems likely to be at least contributory, since in several instances correction of the syndrome has been observed to be accompanied by a reduction in urinary sodium output and a rise in total exchangeable sodium. It is possible that a lesion such as renal artery stenosis may result in the kidney distal to the stenosis receiving inappropriate information about the sodium and water content of the body. In these circumstances the kidney might release increased (and inappropriate) quantities of renin into the circulation, with resultant elevation of the aldosterone secretion rate, lowering of the plasma potassium, and elevation of blood pressure. This rise in systemic arterial pressure would increase the excretion of sodium by the opposite unaffected kidney, while the rise in renin (and angiotensin) might increase the sodium output from both the normal and post-stenotic kidneys. Sodium loss would provide a further stimulus to renin release, with consequent worsening of the syndrome. It seems possible that the increased aldosterone secretion would in these circumstances minimize the negative sodium balance, so preventing the loss of sufficient sodium to render the patient insensitive to the pressor effects of angiotensin. On this hypothesis the cumulative negative sodium balance would remain limited, but would be sufficient to provide a continuing stimulus to renin and thus to aldosterone production.

It is suggested that the correction of a renal artery stenosis alleviates the syndrome by removing the abnormal signal to renin secretion. Unilateral nephrectomy would have a similar effect.

Control of the hypertension by means of drugs could also permit sodium balance to be regained. In some circumstances, a fall in systemic arterial pressure could have the result of stimulating the diseased kidney to produce more renin; such an explanation might apply to those cases in which effective drug treatment of the syndrome is difficult or impossible. Similarly, with a mild or moderately severe renal artery stenosis, the elevation of systemic arterial pressure might be sufficient to turn off the false signal in the post-stenotic kidney. With very severe stenoses this might not be possible until the arterial pressure had risen to heights incompatible with life.

Presumably, multiple small intrarenal arterial lesions, such as occur in various forms of renal disease, may also give a false signal to renin release.

However, not all cases of renal artery stenosis have a raised plasma renin concentration or other features of the hyponatraemic hypertensive syndrome (Fig. 9.4). Generally, the circulating renin level correlates well with the severity of the stenosis and the extent of depression of plasma sodium. In renal artery stenosis, retinal evidence of the malignant phase is usually observed only in the hyponatraemic cases, with raised renin (Fig. 9.4).

An elevated renin is not, however, an invariable accompaniment of the malignant phase; in hypertension due to lesions other than renal artery stenosis retinal and histological evidence of the malignant phase may occur with normal or low plasma renin concentrations.

*Hypertension in Patients with Renal Allografts.* Following total nephrectomy in patients with severe bilateral renal disease, plasma renin falls to extremely low levels. If a renal allograft is subsequently implanted into such subjects, normal or high renin values are observed, the highest renin levels

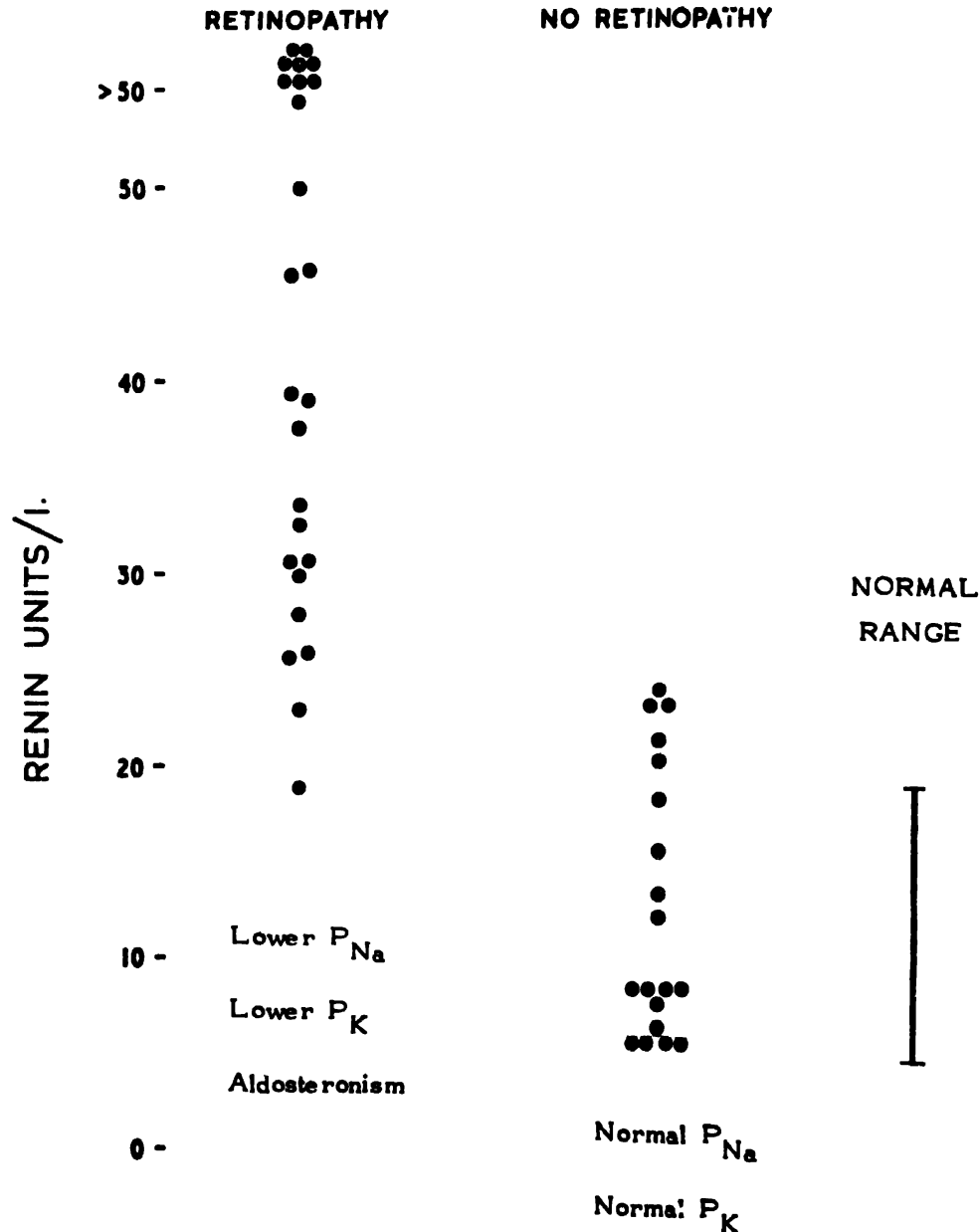
RENAL ARTERY STENOSIS.

FIG. 9.4. Plasma renin concentration in 44 patients with pyelographic, arteriographic and renal functional evidence of unilateral renal artery stenosis. Plasma sodium and renin were inversely correlated. Where measured, plasma aldosterone or aldosterone secretion rate was raised in the cases with high circulating renin levels. "Retinopathy" = papilloedema and/or bilateral retinal haemorrhages and exudates.

generally occurring in the patients with the most severe histological damage in the transplant, in whom the greatest number of rejection episodes have taken place, and who require the largest doses of immuno-suppressive and hypotensive drugs (Brown, Lever & Robertson, 1967).

In patients with renal allografts, in notable contrast to those with renal artery stenosis or other forms of hypertension, no clear inverse relationship between plasma sodium and renin concentration has so far been found.

Summarizing the findings in clinical hypertension, renin is abnormally low in patients with aldosterone-secreting tumours and in some cases of Cushing's syndrome. In pre-eclampsia, the mean plasma renin concentration is low relative to the increase in mean concentration seen in normal pregnancy.

Abnormally high plasma renin concentrations are consistently observed in the hyponatraemic hypertensive syndrome, and frequently in subjects with renal allografts and hypertension. Renin may also be increased to abnormal heights by diuretic treatment or dietary sodium restriction.

Renin is generally normal in cases of phæochromocytoma, aortic coarctation, and "essential" hypertension.

### CLINICAL HYPERTENSION

The following conditions will be considered:

1. Renal artery stenosis.
2. Hypertension due to inappropriate overproduction of aldosterone.
3. Hypertension with hypokalaemia.
4. Phæochromocytoma.

#### Renal Artery Stenosis

The experiments of Carrel (1909) and Janeway (1909) showed that hypertension, in some instances severe and sustained, could be produced experimentally in the dog by the ligation or stenosis of renal artery branches. Goldblatt and co-workers (1934) in the course of more extensive studies, demonstrated persistent hypertension resulting from the application of constricting clamps to both renal arteries.

In recent years advances in the techniques of vascular radiology and in arterial surgery have shown that pathological narrowing of a renal artery in man may be associated with hypertension, which can be corrected either by renal artery reconstruction or by unilateral nephrectomy (Howard *et al.*, 1954; Brown *et al.*, 1960; Spencer *et al.*, 1961).

#### Ætiology of Human Renal Artery Stenosis

Although an atheromatous plaque is the lesion most commonly encountered, renal artery narrowing or occlusion has been reported as a result of a congenital abnormality, angiitis (including syphilitic arteritis), embolism, extrinsic fibrous bands, extrinsic tumours, hydatid cysts, trauma, local aneurysms, fibromuscular hyperplasia, and aortic thrombosis (De Camp & Birchall, 1958; Brown *et al.*, 1960; Wylie & Wellington, 1960; Hunt *et al.*, 1965). Any of these lesions may progress to renal artery thrombosis, in which severe symptoms and marked hypertension may occur (Adams & Newman, 1958; Dollery, Shackman & Shillingford, 1959; Laidlaw, Yendt & Gornall, 1960).

The lesion may be in a branch rather than the main renal artery, and severe hypertension has been observed with segmental renal infarction

following surgical ligation of an aberrant renal artery. There are thus clinical lesions corresponding to the experiments of both Janeway (1909) and Goldblatt and his colleagues (1934).

### Clinical Features

Renal artery stenosis may be encountered at any age (De Camp & Birchall, 1958; Brown *et al.*, 1960).

The association of hypertension with coronary or cerebral arterial lesions, or with intermittent claudication, suggests the possibility of widespread atheroma, which may have caused renal artery stenosis.

Whilst a prolonged and relatively uneventful course does not exclude a renal artery lesion as the basis of hypertension, a rapid increase in arterial pressure, particularly if associated with sudden loin pain, may indicate complete occlusion of the renal artery, with extensive renal infarction (Ben-Asher, 1945; Wainwright, 1949; Adams & Newman, 1959).

Females with renal artery stenosis may be observed to have a raised blood pressure over many years and to present with pre-eclampsia (Landesman, Halpern & Knapp, 1961); it is not uncommon for the lesion in this type of case to be fibromuscular hyperplasia.

Bruits over the loins or abdomen may sometimes be heard (Peart & Rob, 1960; Perloff *et al.*, 1961; Moser & Caldwell, 1962), but are inconstant and often unhelpful diagnostically.

### Diagnostic Procedures

*Intravenous Pyelography.* This is the simplest and probably the most valuable screening test for renal artery stenosis. In a typical case, increased density of contrast, together with smaller pelvicalycine size (indicating diminished urine volume) are found on the side with the lesion (Schlegel, Savlov & Gabor, 1959; Peart, 1959; Brown *et al.*, 1960) (Fig. 9.5). In a small number of patients with severe stenoses and very marked reduction of glomerular filtration rate, contrast density will be low or absent on the affected side. The relative lengths of the kidneys provide a less reliable guide owing to the considerable variation in size and position of the normal kidney.

Several refinements of pyelographic technique have been suggested, such as the performance of the test during water diuresis (Brown *et al.*, 1960), or urea infusion (Amplatz, 1962; Stejskal *et al.*, 1964; Schreiber *et al.*, 1964), so as to reduce contrast density on the normal side, while leaving the post-stenotic pelvis and calyces clearly outlined. Alternatively films may be taken at rapid intervals after the intravenous injection of dye (Maxwell *et al.*, 1964). This often reveals a delay in the appearance of contrast medium on the stenotic side, but it is theoretically possible that the test will occasionally be misleading, since the increased concentration of dye may outweigh the effect of diminished urine volume, and cause the abnormal side to appear first.

Although the value of these various elaborations is limited, standard pyelography is a most useful test for renal artery stenosis, provided it is regarded strictly as a screening procedure, and not as providing a definitive diagnosis. Any asymmetry, especially of contrast density or of pelvicalycine volume, is an indication for more specialized investigation. It is important



FIG. 9.5. Intravenous pyelography in unilateral renal artery stenosis (left-sided).  
(a) IVP in dehydration.



FIG. 9.5. (b) IVP with water load.



FIG. 9.5. (c) IVP in dehydration *after* successful renal artery reconstruction.  
All films taken 15 min after injection of dye.



FIG. 9.6. Pre-operative selective renal arteriogram of patient shown in Fig. 9.5  
illustrating atheromatous stenosis at origin of left renal artery.

to avoid abdominal compression, since this may produce artifactual asymmetry (Stamey *et al.*, 1961; Brown, 1962; Fleming *et al.*, 1965). A pyelographic abnormality was found in 33 of the first 34 cases of renal artery stenosis studied by the present authors, the diagnosis being confirmed by arteriography, together with ureteric catheterization studies or the operative findings. Pyelography may, however, fail to show any asymmetry or other abnormality in the case of a renal artery branch stenosis.

**Radioactive Renography.** This procedure, which was introduced by Taplin and co-workers (1956), involves the intravenous injection of a suitable radio-isotope labelled compound such as  $^{131}\text{I}$ -labelled sodium o-iodohippurate (hippuran) and the detection of its excretion through the kidneys by gamma ray scintillation counters placed over the loins. The physiological basis of the tracings obtained by this technique is discussed by Wedeen and co-workers (1963). Luke and co-workers (1966) have reported results of a very large series of hypertensive patients investigated by this method. Although renography is a simple and safe procedure, it suffers from the disadvantage, in comparison with intravenous pyelography, of not being generally available. Furthermore, although useful as a screening procedure in hypertensive patients, the appearances rarely permit a diagnosis of the nature of the underlying renal disease, and both falsely positive and falsely negative results have been reported (Stewart & Haynie, 1962; Doig *et al.*, 1963; Wax & McDonald, 1964; Fleming *et al.*, 1965).

**Radioactive Scanning.** A more recent development is radioactive scanning of the kidneys (renal "scintiscan") which has the theoretical advantage over the renogram of being able to detect segmental lesions. In this technique radioactive material (e.g.  $^{203}\text{Hg}$ -labelled Chlormerodrin) is injected intravenously, and a renal scan is then obtained by a scintillation counter moving systematically over the lumbar regions (Reba, Wagner & McAfee, 1962; Simmonds & Jones, 1963; Sodee, 1965). Whether the technique of radioactive scanning is capable of detecting areas of ischæmia due to lesions in the smaller intrarenal vessels, which would be missed by pyelography or renography, remains to be determined.

**Renal Arteriography.** Renal arteriography by one or other of the established methods (Sutton, 1964) is invaluable for the localization of renal artery lesions (Fig. 9.6). The morbidity of the test in experienced hands is low (but not negligible). It must, however, be appreciated that a radiographic film taken in one plane cannot provide information on the cross-sectional area of the encroachment on the arterial lumen. Whether or not an apparent stenosis has an effect on the renal circulation cannot therefore be inferred from an arteriogram (although the presence of a post-stenotic dilation implies that it has). Radiological renal artery narrowing does not therefore necessarily indicate renal ischæmia, and renal arteriography alone is a quite inadequate basis for proceeding to operation in the case of a main renal artery stenosis. However, arteriography can reveal lesions in the branches of the renal artery which elude detection by pyelography or ureteric catheterization studies. The segmental blood supply to the kidney is very constant, whether the individual segments are supplied by branches of the main renal artery, or by separate vessels arising directly from the aorta or iliac arteries. Except for the upper and lower poles, renal segments overlap antero-posteriorly;



this means that segmental artery lesions can easily be overlooked if only antero-posterior films are taken. Exposures in two planes are necessary where there is a suspicion of this abnormality (Klapproth, 1959).

*Differential Renal Function Studies by Ureteric Catheterization.* The assessment of the function of each kidney by means of bilateral ureteric catheterization (Howard *et al.*, 1954; Connor *et al.*, 1957) adds precision to the diagnosis of renal and renal arterial lesions. Whilst the early papers emphasized the diagnostic importance of a lowered sodium concentration

	Period	L	R
Urine volume (ml./min.)	11 12 13	2.7 2.0 2.1	9.2 9.0 8.0
Sodium (mEq/l.)	11 12 13	10.0 9.0 6.0	30.0 30.0 22.5
Urine P.A.H. concentration (mg./100ml.)	11 12 13	133.0 138.0 153.0	44.0 39.0 45.0
Urine inulin concentration (mg./100ml.)	11 12 13	375.0 586.0 420.0	130.0 121.0 134.0
P.A.H. clearance (ml./min.)	11 12 13	153.0 118.0 137.0	172.0 148.0 154.0
Inulin clearance (ml./min.)	11 12 13	38.0 40.0 33.0	45.0 41.0 40.0

FIG. 9.7. Results of pre-operative ureteric catheterization studies in patient illustrated in Figs 5 and 6.

in the urine from the post-stenotic kidney, it has been subsequently found that occasionally the urinary sodium concentration is higher on the affected side (Birchall, Batson & Moore, 1958; Brown *et al.*, 1960; Stamey *et al.*, 1961). More reliable guides to a renal artery lesion are comparatively higher urinary inulin, creatinine, and P.A.H. concentrations on the suspected side, observations which correlate with the increased contrast density on I.V.P. (Schlegel, Savlov & Gabor, 1959; Brown *et al.*, 1960; Stamey *et al.*, 1961; Brown, 1962 (Fig. 9.7). These latter findings, together with reduced urine flow and lowered clearances of creatinine, inulin and P.A.H. are the features typical of unilateral main renal artery stenosis. They may also, however, occasionally be found in the absence of a demonstrable renal artery stenosis,

possibly as a result of multiple intra-renal ischaemic lesions (Brown *et al.*, 1960; Brown, 1962).

*Renin and Angiotensin in Renal Artery Stenosis.* As discussed earlier in this chapter, the renin concentration in peripheral venous plasma is not elevated in all cases of renal artery stenosis with hypertension (Brown *et al.*, 1965c), although in the patients with the more severe stenoses, plasma sodium may be low, renin concentration high, and aldosterone secretion rate increased (Brown *et al.*, 1965c; Barraclough *et al.*, 1965).

There is evidence which suggests that the cases with elevated plasma renin and lowered plasma sodium are more likely to respond to surgical correction of the renal artery stenosis than are those with normal renin and electrolytes. If confirmed, this would give renin assays prognostic value in renal hypertension, an assertion which has often been made on insufficient evidence in the past. However, Mulrow (1964) reported successful surgical treatment of renal artery stenosis in patients without detectable elevation of plasma angiotensin, and in whom aldosterone secretion was also normal. Moreover, since renal artery stenosis may be accompanied by histological lesions in the opposite kidney, which may contribute to a raised plasma renin concentration, not all patients with high circulating renin would be expected to respond to surgical correction.

*Angiotensin Infusion Tests.* Kaplan & Silah (1964) approached this problem indirectly. Reasoning that the pressor responsiveness to infused angiotensin would be inversely related to its circulating level (see page 281), they suggested that cases of renal hypertension suitable for surgical treatment would show little rise in arterial pressure when angiotensin was administered. Kaplan & Silah claimed that if this test was applied to a group of hypertensive patients (without severe retinopathy) it might serve to differentiate cases of surgically-treatable renal artery stenosis from other forms of hypertension. Many workers have been unable to confirm this (Wax, 1965; Morgan, 1965; Breckenridge, 1965; Blair, 1965; Laragh, 1966; Page, 1966), and the test may not be without danger (Laragh, 1966). Others, however, have found the procedure useful diagnostically (Hocken, Kark & Passavoy, 1966). The method presupposes elevated angiotensin blood level in cases where renal artery stenosis is responsible for hypertension, a point which, as noted above, remains to be established. In principle, if elevated circulating renin or angiotensin should have prognostic importance in renal hypertension, direct assay would be more reliable.

Rob & McDonald (1965) suggested that angiotensin infusions in combination with ureteric catheterization studies might aid diagnosis, in that these could produce antidiuresis in the post-stenotic kidney, with diuresis on the opposite side. This suggestion is based on a misconception, however, since it has been found that in the majority of cases of unilateral renal artery stenosis investigated in this way, both kidneys behave in a similar fashion during angiotensin infusions (Brown & Robertson, 1962; Brown, Matthew & Robertson, 1964a).

### **Treatment of Renal Artery Stenosis**

The upsurge of enthusiasm for renal artery reconstructive operations in the early nineteen-sixties has been followed by a more cautious therapeutic

approach, although there is no doubt that in suitable cases excellent results may be obtained. Several groups have reported a remarkably high rate of success (e.g. Morris *et al.*, 1962; Rob, 1961), although others have been apparently less fortunate (Dustan *et al.*, 1963; De Graeff & Struyvenberg, 1966; Sokolow *et al.*, 1966). In a recent study, Morris and co-workers (1966) found a 21 per cent. incidence of recurrence of hypertension in patients operated upon for renal artery stenosis.

A few points are, therefore, worth brief emphasis. Not all radiologically apparent stenoses affect renal blood flow or pulse pressure; nor does the demonstration that a stenosis is severe enough to produce a measurable change in renal function imply that the stenosis is necessarily the immediate cause of the raised blood pressure. Reconstructive procedures are technically difficult in a vessel of the size and situation of the renal artery; surgical interference can readily convert a stenosis into a thrombosis, with consequent worsening of the situation. Moreover, a post-stenotic dilation may be associated with extreme friability of the arterial wall. In either case unilateral nephrectomy may be an inevitable result of surgical interference. Consequently it is imperative to ascertain before operation whether or not adequate function remains in the opposite kidney.

The experimental observations of Wilson & Byrom (1941) suggested that severe histological damage in the opposite kidney might perpetuate hypertension after removal of a renal artery clip. The case reported by Thal, Grage & Vernier (1963) seems to provide the clinical counterpart to these observations. This patient's blood pressure remained high after reconstruction of the stenosed right renal artery. Three months later the function of this kidney was much better than the function of the unoperated left kidney, and the blood pressure was subsequently returned to normal by removing the previously undisturbed left kidney. Histological examination demonstrated that "the walls of the small and medium sized arteries" in the excised kidney were "almost uniformly thickened", while the renal artery was "essentially normal". Stamey (1965) has recently confirmed that marked reduction in the P.A.H. clearance (less than 250 ml./min./1.73 m.<sup>2</sup>) on the side opposite the stenosis indicates that renal arterial surgery is unlikely to alleviate the hypertension.

In atheromatous renal artery stenosis the natural history of the disease is such that the lesion may recur after operation, or that atheroma may involve the opposite renal, the coronary, or the cerebral arteries.

Finally, the presence of a renal artery lesion does not preclude effective medical treatment of the hypertension (Brown *et al.*, 1960; Dustan *et al.*, 1963).

#### **Hypertension with Autonomous Oversecretion of Aldosterone: Primary Hyperaldosteronism**

The condition generally known as "primary aldosteronism" was described by Conn (1954, 1955), Mader & Iseri (1954; 1955), Cope & Llaurodo (1954), Llaurodo (1955) and Milne, Muercke & Aird (1956). The syndrome has been reviewed recently by Relman (1963), Conn, Knopf & Nesbit (1964) and Luetscher (1964).

**Clinical Features.** Although these patients may present with symptomless

hypertension, they quite commonly experience muscular weakness, which sometimes proceeds to intermittent paralysis, nocturia, polydipsia, headache, paræsthesiæ and tetany. The neuromuscular symptoms seem to be more common in females. Œdema is comparatively rare (Conn *et al.*, 1964a).

Typically, hypertension is accompanied by hypernatræmia, hypokalæmia, increased total exchangeable sodium, decreased total exchangeable potassium, raised plasma and extracellular fluid-volume, and extracellular alkalosis. Although some cases with normal electrolytes have been reported (Luetscher, 1964; Conn *et al.*, 1965), there are probably few instances in which repeated and careful measurements of plasma sodium and potassium do not reveal any abnormality.

The secretion rate, plasma concentration, and urinary excretion of aldosterone are typically elevated.

Although comparatively uncommon, retinal hæmorrhages and exudates (Delorme & Genest, 1959; Brill, Creamer, Mills & Pullan, 1960; Luetscher, 1964), papillœdema (Kaplan, 1963) and fibrinoid arteriolar lesions (Brown *et al.*, 1964d) have been reported.

**Pathology.** In the great majority of cases, a single benign adrenocortical adenoma is responsible; occasionally multiple tumours are found. Rarely, the neoplasm is malignant (Foye & Fechtmeir, 1955; Santander, Gonzalez & Suarez, 1965; Crane, Harris & Herber, 1965). In some cases the syndrome has apparently occurred in the absence of an adrenal tumour (Bartter & Biglieri, 1958; Relman, 1963; Ross, 1965).

**Diagnosis.** In many instances, the disease can be diagnosed by careful study of the clinical and biochemical features outlined above. Relman (1963) has emphasized that accurate metabolic balance studies may allow aldosterone measurements to be omitted, although these clearly add precision to the diagnosis.

*The Renin-angiotensin System in Primary Hyperaldosteronism.* Several workers (Laragh, Cannon & Ames, 1963; Goldberg & McCurdy, 1963; Brown *et al.*, 1963a, b, 1964b, c; Kirkendall, Fitz & Armstrong, 1964) have appreciated that assessment of the renin-angiotensin system might help to distinguish cases of this type from other forms of hypertension with hypokalæmia and increased aldosterone production. This, in general, has been borne out. Thus, plasma renin concentration is either abnormally low or in the lower range of normal in untreated cases, and rises after the tumour is removed (Brown *et al.*, 1963a, 1964d, 1965a, c).

Circulating angiotensin has usually been undetectable, although this is not an invariable finding (Biron *et al.*, 1962; Morris, Robinson & Scheele, 1964).

The results of "renin-activity" estimations have been variable. Yoshinaga and co-workers (1963), using a modification of the method of Helmer (1964), found values in the upper normal range. Kirkendall, Fitz & Armstrong (1964), using a similar technique, obtained low or undetectable values, which rose to normal after removal of the tumour. Conn and co-workers (1964b) and Meyer and co-workers (1965) later obtained similar results to those of Kirkendall, Fitz & Armstrong (1964).

These various methods can thus support the diagnosis of aldosterone-secreting tumours, although taken alone they are not diagnostic. As dis-

cussed earlier, renin would be expected to be depressed whenever sodium retention was caused by mechanisms other than the renin-angiotensin system. Sutherland, Ruse & Laidlaw (1966) have recently described cases of hypertension with hypernatraemia, hypokalaemia, low plasma renin activity, and increased aldosterone secretion. Adrenal tumours were not found, and these patients were stated to differ from Conn's syndrome also in that the abnormalities were corrected by dexamethasone administration. Moreover, plasma renin concentration may be elevated to normal in cases of primary hyperaldosteronism by diuretic therapy or potassium loading (Brown *et al.*, 1963a, 1964b, 1965a; Meyer *et al.*, 1965). Conn, Cohen & Rovner (1964b) emphasized that in primary hyperaldosteronism renin-activity may increase less briskly than normal in response to sodium restriction or to assumption of the upright posture. While these manœuvres may help, it is clear from a later paper (Conn *et al.*, 1965) that they do not always permit a clear distinction from normal. Indeed, despite one interesting report by Conn and his colleagues (1965), it remains to be demonstrated that assays of renin or angiotensin have diagnostic advantage over repeated and careful measurements of plasma electrolytes. It is important that plasma, as opposed to serum, is used for potassium estimation. The serum concentration of potassium is much more variable than the plasma concentration, which it may exceed in the same specimen of blood by as much as 0.8 mEq (Pfleiderer, Otto & Hardegg, 1959; Whitfield, 1966). Diagnostic hypokalaemia might well be obscured if measurement is made using serum.

Circumstantial evidence suggests that the granularity of the juxtaglomerular apparatus is proportional to the level of circulating renin (Hess & Gross, 1959; Tobian, 1960a, b; Fisher & Klein, 1963; Hartroft, Sutherland & Hartroft, 1964). It follows that hypogranularity would support the diagnosis of aldosterone-secreting tumour (Itskovitz *et al.*, 1963), and this has been demonstrated on renal biopsy material in several cases (Cohen *et al.*, 1965; Brown *et al.*, 1965a; Meyer *et al.*, 1965).

**Radiological Localization.** The adrenocortical tumour may sometimes be demonstrated arteriographically (Brown *et al.*, to be published) and where the diagnosis is suspected, contrast medium should be injected into the aorta above the renal arteries sufficiently high to fill the arteries supplying the adrenal glands.

Adrenal venography, in which contrast medium is injected into an adrenal vein through a radio-opaque catheter introduced via the femoral vein, may also show the tumour (Bucht *et al.*, 1964; Starer, 1965). The left adrenal vein is easier to catheterize since it joins the renal vein on that side.

In some cases retroperitoneal air insufflation may be helpful, but it can be misleading, and is also more dangerous and uncomfortable for the patient than arteriography or venography.

## Treatment

The hypertension and other abnormal features usually, but not invariably, respond to removal of the tumour (Conn, Knopf & Nesbit, 1964a). Occasionally the tumour is small, and may not be found until both adrenals have been removed and sectioned, or until the blood supply to both adrenal

glands has been jeopardized in the search (Relman, 1963; Slaton & Biglieri, 1965).

An alternative form of therapy which avoids removal of both adrenals in these instances, is the prolonged administration of oral spironolactone. This treatment can restore the electrolytes, blood pressure and renin concentration to normal, while leaving aldosterone secretion undiminished (Brown *et al.*, 1963a, b, 1964d, 1965a; see also Part I). Care is needed, however, when using these drugs, since the rate of secretion of aldosterone by the tumour may be capable of little or no variation, and aldosterone secretion by the remainder of the adrenal cortex may be suppressed. Large doses of spironolactone might in these circumstances result in sodium depletion and postural hypotension, particularly in the early stages of treatment.

Spironolactone therapy may also cause enlargement and tenderness of the breast (Smith, 1962; Brown *et al.*, 1965a). This resolves if treatment is withdrawn. Epigastric discomfort occurs in some patients, but may be averted by administering the drug after meals; constipation and dysmenorrhoea also occur.

Treatment with oral spironolactone can also help to establish a diagnosis of primary hyperaldosteronism where this remains in doubt, correction of the hypertension and electrolyte abnormalities strongly favouring the diagnosis.

### Differential Diagnosis of Hypertension with Hypokalæmia

Hypokalæmia is a relatively common finding in patients with hypertension. The following principal categories—in their approximate order of frequency—require consideration:

1. Administration of benzothiadiazine drugs to hypertensive patients.
2. "Hyponatræmic" syndrome of severe hypertension with increased aldosterone secretion.
3. Aldosterone-secreting adenoma.
4. Excessive cortisol secretion (see chapter 5):
  - (a) Cushing's syndrome.
  - (b) ACTH-producing tumour.
5. Potassium-wasting renal disease.
6. Excessive use of purgatives or liquorice.
7. Congenital adrenal hyperplasia.
8. Familial tubular syndrome (Liddle).

1. *Benzothiadiazine* administration may produce hypokalæmia and often, although not invariably, hyponatræmia, increased aldosterone secretion and a rise in  $\text{TCO}_2$ . Plasma renin concentration rises as a result of sodium loss. Similar, but more severe, changes may also occur if these drugs are given inadvertently to patients with primary hyperaldosteronism, when dangerous potassium deficiency may result.

The use of benzothiadiazines in hypertension may in any event effectively obscure the diagnosis, and some effects can persist for at least 3–4 weeks after stopping treatment.

2 and 3. *The Differential Diagnosis of Primary Hyperaldosteronism and Hypertension with Secondary Hyperaldosteronism.* The association of hyper-

tension and hypokalaemia was noted by de Wesselow & Thomson (1938). Their studies, and those of Holten & Petersen (1956), Fitzgerald and co-workers (1957), Wrong (1957), Laidlaw, Yendt & Gornall (1960), Wrong (1960) and Gowenlock & Wrong (1962), showed clearly that even where associated with increased aldosterone excretion or secretion, these cases were not necessarily instances of primary adrenal adenoma, but that many were examples of a "hyponatraemic hypertensive syndrome", in which adrenalectomy might be inappropriate and unrewarding.

In this hyponatraemic syndrome (see page 278), the increased aldosterone secretion may well be secondary to the hypertension (Fitzgerald *et al.*, 1957; Brown *et al.*, 1966). Plasma sodium concentration and plasma volume are usually low, and the urinary sodium output may be inappropriately high for the low plasma sodium level. Frequently, the hypertension is in the malignant phase, and in many instances an intrarenal lesion, or a renal artery stenosis, is present. Other aspects of the differential diagnosis have been discussed in previous sections.

4. *Excessive Cortisol Secretion* (see Chapter 5).

5. *Potassium-wasting Renal Disease*. Cases of this type, which include cases of chronic pyelonephritis, polyarteritis, renal tubular acidosis, and the Fanconi syndrome, have on occasions been confused with primary hyperaldosteronism, since hypertension, potassium depletion and increased aldosterone secretion occur in both. The most important differential diagnostic points are that in potassium-wasting renal diseases the excretion of sodium is also excessive, and acidosis, raised blood urea and decreased plasma and exchangeable body sodium commonly occur, in contrast to the opposite findings in primary hyperaldosteronism.

6, 7, 8. Of the other forms of hypokalaemic hypertension listed, Liddle's syndrome requires special mention. This is a familial renal disorder affecting both sexes, occurring in successive generations, and characterized by hypertension, hypokalaemic alkalosis and negligible aldosterone secretion (Liddle, Bledsoe & Coppage, 1963). The condition is apparently due to a tubular abnormality which causes excessive retention of sodium and inappropriate excretion of potassium, despite the virtual absence of mineralocorticoids. It can be differentiated from primary hyperaldosteronism by the failure of a 3-4-day course of spironolactone to influence sodium and potassium excretion. In Liddle's syndrome, however, the excretion of these electrolytes is reversed, and the plasma electrolytes and blood pressure are returned to normal by treatment with triameterene, an agent which apparently acts directly on the renal tubular cells.

### **Phæochromocytoma**

Phæochromocytoma (chromaffinoma) is the name applied to a tumour of chromaffin tissue, the cells of which assume a yellow-brown colour when treated with chrome salts. These tumours, which in 80 per cent of cases, are found in, or close to, the adrenal glands, vary in size from tiny nodules to large masses weighing 3-4 kg.

The clinical features will be considered in four categories:

1 Typical paroxysmal hypertension.

2, Persistent hypertension with or without paroxysms.

3. Attacks of *hypotension*.
4. Renal artery stenosis and phæochromocytoma.

*Typical Paroxysmal Hypertension.* During the paroxysms, the plasma concentration of adrenaline and/or noradrenaline rises (Beer, King & Prinzmetal, 1937) and the common complaints therefore include headache, palpitations, vomiting, dyspnœa, pallor, substernal discomfort, abdominal pain, tremor and nervousness (Graham, 1951). A large increase in both the systolic and diastolic blood pressure usually occurs (e.g. from 130/90 to 200/120), but in some patients the diastolic pressure remains relatively unchanged. Similarly, the pulse rate is often increased, although bradycardia sometimes occurs. These differences might be determined by the ratio of adrenaline to noradrenaline released by the tumour, since similar alterations have been observed during intravenous infusion of solutions containing varying proportions of adrenaline and noradrenaline (De Largy *et al.*, 1950). An increase in the urinary excretion of dopamine (the immediate precursor of noradrenaline) has also been recorded in patients with phæochromocytoma. Although less active, this amine shares some of the pharmacological actions of adrenaline and noradrenaline (Horwitz, Fox & Goldberg, 1962; Allwood, Cobbold & Ginsberg, 1963) and may therefore contribute to the clinical features in some patients.

Swelling at the root of the neck during the attacks is an interesting feature mentioned in the first full clinical description of phæochromocytoma (Labbé, Tinel & Doumer, 1922). A similar swelling has been noted during noradrenaline infusions in man, and attributed to thyroid enlargement due to a combination of increased blood flow and œdema (Mowbray & Peart, 1960).

The cause of the profuse sweating which commonly occurs during the paroxysms is less certain. A new explanation is suggested by recent observations of a patient from whom two phæochromocytomas were removed. Before operation, intravenous infusion of adrenaline and noradrenaline provoked a facial flush and moderate sweating of the face, palms and axillæ, while post-operatively similar infusions led to the normal response of skin pallor without sweating. These observations raise the possibility that the flushing and sweating seen in this patient were provoked by a substance (or substances) released from the tumour in response to adrenaline and noradrenaline (Brown & Rawson, 1965, unpublished). It might be relevant to recall that the flushes provoked by injections of adrenaline in patients with the carcinoid syndrome (Robertson, Peart & Andrews, 1962) are accompanied by an increase in hepatic venous kinin concentration (Oates *et al.*, 1964) which is probably due to the release of kallikrein from the tumours. During the infusion of bradykinin in one patient with a carcinoid tumour, facial flushing and sweating were observed (Grahame-Smith, 1965, personal communication).

*Persistent Hypertension.* Although the blood pressure remains normal between paroxysms in some patients with a phæochromocytoma, in others the hypertension is persistent. A history of typical paroxysmal symptoms should raise the possibility of a phæochromocytoma in either group, but in some cases paroxysmal symptoms are atypical or absent. A history of excessive sweating, or of mottling or tingling in the skin, and the observation of a



raised body temperature or of glycosuria should suggest the diagnosis of a phæochromocytoma even in the absence of the more classical symptoms (Smithwick *et al.*, 1950). Occasionally, patients with a phæochromocytoma and persistent hypertension deny any of these symptoms (Thorn, Hindle & Sandmeyer, 1944; Richardson, Ross & Turnbull, 1955; de Graeff & Horak, 1963), so that this diagnosis should be considered in all cases of hypertension of unknown cause. The suspicion is strengthened if the patient has multiple neurofibromatosis, von Hippel Lindau's disease (retinal angioma and cerebellar hæmangioblastoma), Sturge-Weber syndrome (hæmangiomata of face, meninges and brain), tuberose sclerosis, thyroid carcinoma, or a family history of phæochromocytoma, since the incidence of phæochromocytoma is increased in each of these circumstances (Brown, 1965). Persistent hypertension is said to be more common in children with phæochromocytomas than in adults (Hume, 1960).

Although paroxysms of hypertension are clearly related to the release of adrenaline and noradrenaline from the tumours, the cause of persistent hypertension is less certain, since the plasma concentration of pressor amines was normal in one patient of this type (Kvale *et al.*, 1956). An attempt to produce a sustained rise in blood pressure in the rabbit by prolonged intravenous infusion of adrenaline and noradrenaline was unsuccessful (Blacket, Pickering & Wilson, 1950). Pickering (1955) has suggested that persistent hypertension in patients with a phæochromocytoma might be a legacy of the effects produced in the cardiovascular system by paroxysmal attacks.

*Attacks of Hypotension.* In contrast to the common presentation with intermittent or persistent hypertension, a marked fall in blood pressure is sometimes observed in patients with a phæochromocytoma. The diagnosis should not be difficult when the fall of blood pressure follows a typical hypertensive episode. Patients with phæochromocytoma are, however, prone to develop severe hypertensive attacks during anæsthesia. If this occurs when a tumour has not been suspected, the relevance may be missed, and the post-operative fall in blood pressure then incorrectly attributed to other causes.

The mechanism of the hypotension remains obscure. It might be related to a reduced cardiac output following a fall in plasma volume; to pooling of blood in peripheral vessels after the sudden removal of vasoconstrictor tone; to the action of an unrecognized vasodilator substance (possibly a kinin); to corticosteroid deficiency, or to saturation of arteriolar walls with noradrenaline. These possibilities are discussed in more detail elsewhere (Brown, 1965).

*Renal Artery Stenosis and Phæochromocytoma.* The combination of unilateral renal artery stenosis with a phæochromocytoma in the adjacent adrenal gland has been reported in at least three patients. At operation in two of these cases, the renal artery was stretched around the tumour (Rosenheim *et al.*, 1963), but in the third patient (Garrett *et al.*, 1965), as well in an unpublished case (Brown, 1965) the phæochromocytoma was not compressing the main renal vessels. While this association may be quite fortuitous, the combination of two uncommon lesions within the same patient raises the possibility of a causal relationship. Garrett and co-workers (1965) suggested that the phæochromocytoma might lead, in an undefined way, to changes in the wall of the adjacent renal artery. The opposite sequence, renal artery

stenosis leading to the development of a phæochromocytoma, seems at least as likely, since the plasma concentration of renin and angiotensin is increased in some patients with renal artery stenosis (see p. 289), and infusion of angiotensin stimulates the adrenal medulla at least in some species (see p. 277). It is possible, therefore, that prolonged exposure of the adrenal medulla to high levels of circulating angiotensin might lead to the development of a phæochromocytoma.

### Pharmacological Tests for Phæochromocytoma

These are of two types and involve the use of:

1. Drugs which stimulate the release of catechol amines and therefore provoke attacks similar to the spontaneous paroxysms, e.g. histamine and tyramine. The mechanism of the release of catechol amines from the adrenal medulla by histamine has been investigated by Staszewska-Barczak & Vane (1965).

2. Drugs which oppose the action of catechol amines, e.g. phentolamine and phenoxybenzamine. The usefulness of these is restricted to patients with persistent hypertension, or investigation during a spontaneous hypertensive paroxysm.

Since misleading effects sometimes occur, these tests have been largely superseded by the more precise biochemical and pharmacological procedures discussed below. However, provocative tests may be useful when other results are equivocal.

**Histamine Stimulation Test.** An intravenous glucose infusion is set up and the blood pressure and pulse rate are recorded at  $\frac{1}{2}$ –1 minute intervals. When a steady state is reached, injections of histamine (starting with 10  $\mu$ g; maximum dose 100  $\mu$ g) are given into the infusion tubing. A rise in systolic blood pressure of more than 40 mm Hg within 3 minutes strongly suggests the presence of a phæochromocytoma. Phentolamine (10 mg) and propranolol (3–10 mg) should be available for immediate intravenous injection if a severe attack occurs in response to histamine.

**Tyramine Stimulation Test.** Engelman & Sjoerdsma (1964a) recently reported that the pressor response to intravenous tyramine was enhanced in patients with a phæochromocytoma. A rise in systolic blood pressure greater than 20 mm Hg following the intravenous injection of 1 mg (or less) of tyramine supports the diagnosis of phæochromocytoma. This test is based upon the theory that the pressor response to tyramine is due to the liberation of noradrenaline (or similar substances) from tissue stores (Burn & Rand, 1958). Since the tissue stores of adrenaline and noradrenaline can be increased by injection of these amines (Stromblad & Nickerson, 1961) it is likely that a similar increase in catechol amine stores occurs in patients with a phæochromocytoma, and that large quantities may be released by tyramine. Misleading results may be obtained if the patient is being treated with reserpine, methyldopa or monoamine oxidase inhibitors; treatment with reserpine depletes the stores of noradrenaline and inhibits the pressor response to tyramine (Burn & Rand, 1958), while the response is enhanced by methyldopa (Pettinger *et al.*, 1963) and by monoamine oxidase inhibitors (Asatoor, Levi & Milne, 1963).

### Laboratory Investigations

**Urinary Excretion of Catechol Amines.** An increased urinary excretion of adrenaline and noradrenaline in patients with phæochromocytomata was first reported in 1950 (Engel & Euler). Since catechol amines are unstable in alkaline solution, the estimation is performed on an aliquot (100–200 ml) of the urine collected during a 24-hour period into a bottle containing 10 ml concentrated HCl. The amines are adsorbed on alumina, eluted and then estimated by pharmacological or fluorimetric methods.

**Urinary Metabolites of Adrenaline and Noradrenaline.** The major metabolic breakdown of adrenaline and noradrenaline proceeds via the meta-

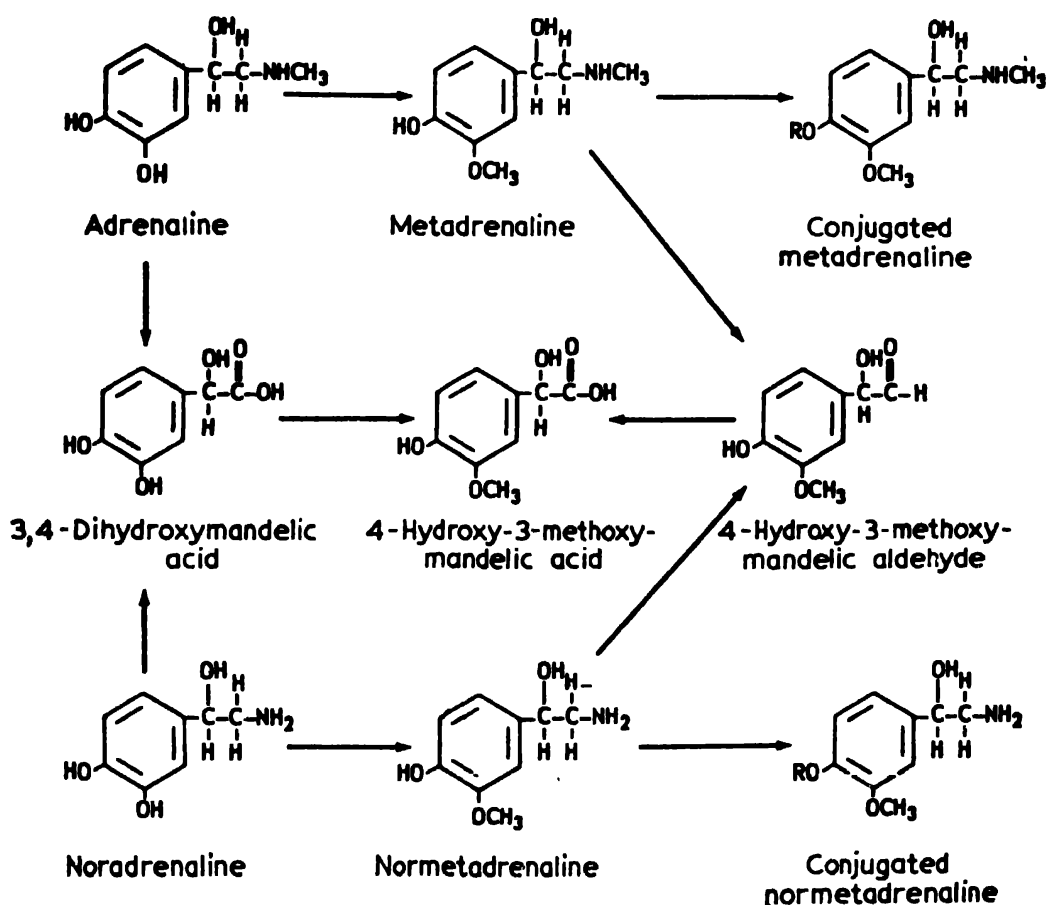


FIG. 9.8. Metabolic pathways of adrenaline and noradrenaline.

nephries (metadrenaline and normetadrenaline) to 4-hydroxy-3-methoxy-mandelic acid (vanillylmandelic acid or VMA) (Fig. 9.8). (Sandler, 1963). These metabolites appear in the urine in greater quantities than the parent amines, and increased excretion occurs in patients with phæochromocytomata. An elevated output of homovanillic acid (the major metabolite of dopamine, the precursor of noradrenaline) may be more common in patients with malignant phæochromocytomata (Robinson, Smith & Whittaker, 1964; Sato & Sjoerdsma, 1965).

The diagnosis of phæochromocytomata can usually be established when any one of the foregoing investigations is performed in a reliable laboratory.

Occasionally an equivocal result is obtained and it is then advisable to include one of the alternative measurements. If the diagnosis remains in doubt, the plasma level or urinary excretion of adrenaline and noradrenaline should be estimated during a spontaneous or a provoked attack.

### **Localization of Phæochromocytomata**

The tumours are found in the abdomen in approximately 95 per cent and within the adrenal glands in about 80 per cent of cases. More precise pre-operative localization usually depends upon retroperitoneal pneumography and aortography, which are reasonably safe procedures when carried out by experienced radiologists. However, severe and occasionally fatal hypertensive attacks have occurred during these investigations and it is, therefore, advisable to treat the patient with phenoxybenzamine and propranolol during the preceding 2–3 days.

If the tumour is not located by these procedures, it may be found by estimating the catechol amine concentration in blood taken from the venæ cavæ at various sites through a catheter inserted under local anæsthesia into a peripheral vein and guided under radiological control. In the absence of a spontaneous attack, an elevated catechol amine concentration at a particular sampling site indicates the entry of blood from veins draining the tumour.

### **Pre-operative, Operative and Post-operative Medical Management**

The correct treatment is to remove the tumour as soon as possible. Meanwhile the symptoms can be diminished or abolished by oral treatment with phenoxybenzamine ( $\alpha$ -receptor blocker: 20–60 mg/day) and propranolol ( $\beta$ -receptor blocker: 40–60 mg/day). Although cardiovascular crises are probably less frequent in patients prepared for operation in this manner, the release of amines from the tumour may still cause severe hypertension or cardiac arrhythmia. These complications are particularly prevalent during induction of anæsthesia and when the tumour is being dissected: hypertension may then be reversed by phentolamine, and arrhythmias by propranolol given intravenously. After excision of the tumour profound hypotension may occur. This is treated by intravenous infusion of noradrenaline (4 mg in 1 litre 5 per cent glucose containing 10 mg ascorbic acid as a preservative).

Since the patient may harbour more than one tumour, the urinary excretion of catechol amines or their metabolites, should be estimated again post-operatively.

### **Prolonged Medical Treatment**

A prolonged course of treatment with phenoxybenzamine has been found to be beneficial in a group of patients who were considered unfit for surgical treatment (Allen *et al.*, 1951; Engelman & Sjoerdsma, 1964b). An alternative therapy for such cases stems from the search for compounds which interfere with the metabolic synthesis of catechol amines (Fig. 9.9).  $\alpha$ -methyl dopa (Aldomet), a decarboxylase inhibitor capable of interfering with the conversion of dopa to dopamine, was observed to reduce the excretion of catecholamine metabolites in several cases of phæochromocytoma, but treat-

ment had to be stopped because side effects due to postural hypotension were intolerable (Engelman & Sjoerdsma, 1964b).

Recent observations suggest that  $\alpha$ -methyl tyrosine may be useful in the

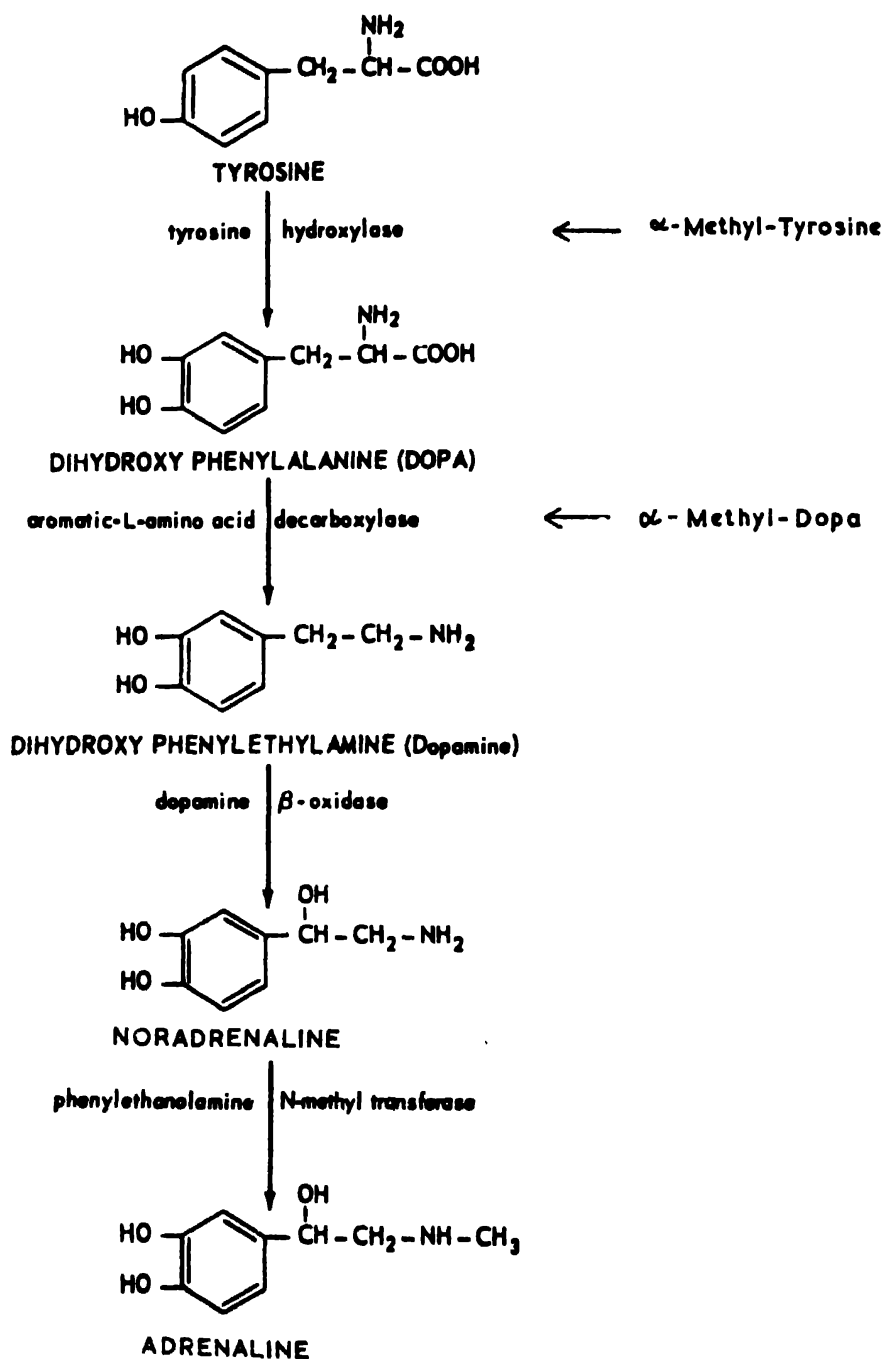


FIG. 9.9. Sites of action of  $\alpha$ -methyl-tyrosine and  $\alpha$ -methyl-dopa.

medical treatment of phæochromocytoma. During treatment with this compound, which inhibits the hydroxylation of tyrosine to dopa, the blood pressure was lowered, and the excretion of noradrenaline, adrenaline and their metabolites was reduced (Sjoerdsma *et al.*, 1965).

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## CHAPTER 10

# LEUKÆMIA

by

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AT the time of writing, 1967, the causes of leukæmia and allied malignancies of the lymphoid tissues are unknown. Research on the nature of leukæmia can be divided broadly into studies in man and studies in animals. Leukæmias in experimental animals and those occurring in man have many points of dissimilarity, which must be realized when correlating the knowledge gained from the study of mice to the clinical problems of human leukæmia.

The majority of the reliable statistics about the behaviour of the disease and the effects of therapy came from hospitals that run large leukæmia clinics and received their patients from a wide area. There are many arguments in favour of concentrating leukæmic patients in special centres; greater therapeutic risks may be taken if facilities are available to provide specialized supportive measures during the periods of severe bone marrow hypoplasia.

### Incidence

The incidence of leukæmia can be judged from the Registrar General's returns for England and Wales. Table 10.I shows these figures for 1963, 1964 and 1965. Leukæmia is responsible for a fairly constant fraction of the total deaths and its incidence is comparatively low compared with cardio-vascular disease and the other forms of malignancy such as carcinoma of the stomach or bronchus. This relatively low frequency of leukæmia contributes to the slow progress made in assessing new therapies.

TABLE 10.I

INCIDENCE OF LEUKÆMIA AS A CAUSE OF DEATH IN ENGLAND AND WALES\*

<i>Year</i>	<i>All causes</i>	<i>Leukæmia</i>	<i>Percentage due to Leukæmia</i>
1963	572,868	2,830	0.49
1964	534,737	2,867	0.53
1965	549,379	2,860	0.52

\*From the Registrar General's reports

### Epidemiology

**Geographical and Seasonal Variation.** In both Britain and the United States there have been reports of abnormally high incidence of leukæmia

in certain districts over a particular period of time (Heath & Hasterlik, 1963; Wood, 1960). In Britain leukæmia in young people appears to be more prevalent at certain times of the year (as indicated by the month in which cases are first admitted to hospital) (Knox, 1964). May and June produce one peak and December and January another. It has since been shown that a similar phenomena occurs in chronic lymphatic leukæmia. The populations studied have been small and of course there is an unknown interval between the true beginning of leukæmia and the moment the patient is aware of his disease, and also a variable period prior to admission to hospital. This is particularly so in chronic lymphatic leukæmia where many months, perhaps years may elapse before the patient reports symptoms or is accidentally discovered to have the disease.

The high incidence of leukæmia in an area, a "leukæmic cluster", is of great interest in view of a possible infective (viral) ætiology. The relation between geographical location, season and the incidence of leukæmia will require to be checked for many other areas of the world before any firm conclusion can be reached.

**Leukæmia in Twins.** A possible genetic factor in leukæmogenesis has been reported by MacMahon & Levy (1964) following the examination of the records of 72 sets of twins in which at least one twin had leukæmia in childhood. In five sets both twins were affected and there was evidence to suggest that these were monozygotic twins. They were able to find reference in the literature to 25 cases of leukæmia occurring in both twins. Their observations suggest that both twins may have been exposed to a leukæmogenic factor at the same time and that this may have been during gestation.

### Ætiology of Leukæmia

**Radiation** is the most firmly established leukæmogenic agent in man. The relation between radiation dosage and the incidence of leukæmia has been studied in the survivors of the atomic bomb explosions at Hiroshima and Nagasaki. In these populations the incidence of leukæmia has been approximately five times greater than that which would have been expected to arise spontaneously. There was a higher incidence among children than adults. The incidence of leukæmia began to rise about 18 months after the explosions and reached its peak 4–7 years later and had returned almost to normal after 15 years. The incidence has been approximately linear to the dose received above 100 rads; below this level the data were inadequate for any prediction. Above 100 rads, the incidence of leukæmia has been calculated to be 1–2 cases per year per rad per  $10^6$  population at risk (Cronkite, Moloney & Bond, 1960).

Patients with ankylosing spondylitis who have been given a high dosage of X-rays to the spine have a raised incidence of leukæmia (Court, Brown & Doll, 1957) and there is evidence for an increased incidence of leukæmia in children who have had X-ray therapy for thymic tumours. Furthermore, the ingestion of thorotrast and radioactive iodine is associated with an increased susceptibility to leukæmia. Treatment of polycythemia rubra vera with  $^{32}\text{P}$  increases the tendency to leukæmic change. Watkins, Fairley & Bodley Scott (1967), in reporting their own experiences and summarizing the literature, conclude that acute leukæmia is a very rare complication of polycythæmia

rubra vera unless  $^{32}\text{P}$  or DXR have been used, when the incidence usually exceeds 15 per cent.

The evidence concerning the leukæmogenic effects of, low dose irradiation from diagnostic X-rays, diagnostic tests involving radioisotopes and fall-out from nuclear bomb explosions is uncertain. There is no information as to the possible threshold level of radiation leukæmogenesis in man. Even after high doses of radiation only a comparatively few of those exposed eventually develop leukæmia, and this indicates that radiation *per se* is not invariably leukæmogenic.

**Viruses and Mycoplasmas.** During the past 50 years it has been unequivocally demonstrated that leukæmia in certain laboratory and domestic animals is initiated by viruses. Those viruses capable of inducing leukæmia in specific strains of mice and those causing avian leucocytosis have received the greatest attention. Harris (1965) has reviewed the main features of these viruses. The viruses capable of altering mammalian cells have a considerable spectrum of effects; some lead to degeneration alone, some to proliferation and degeneration, some cause benign proliferation only and others induce malignant change.

In this chapter the work on animals will be considered only in its relation to research on a possible viral ætiology in human leukæmia. A viral ætiology of leukæmia in certain species warrants the search for such an ætiology in human leukæmia. The study of murine and avian viruses is strewn with pitfalls and the diseases produced in the animals have no strict counterparts in human leukæmias, and certain essential facts must be borne in mind when correlating animal and human research. Frequently in murine leukæmias the infectivity of a leukæmogenic virus is profoundly influenced by the strain of mouse that is inoculated. Large amounts of virus are usually present in the cells and blood of viral induced leukæmias in mice and chickens. No difficulty is encountered in demonstrating virus, for example, in chicken myelosis or the Friend murine leukæmia. In certain high inbred strains of mice there is a very high incidence of leukæmia. This has been shown by Gross (1965) to be due to a vertical transmission of leukæmogenic virus from one generation to the next in the sperm or ovum.

The search for viruses in human leukæmia is often conducted by indirect methods, for those methods which have been so successful in animals, i.e. injecting the agent into newborn or compatible animals of the same species as that bearing the suspected virus, is obviously impossible. Viruses can be studied for their effects on tissue cultures (especially on human foetal cells), for their antigenicity and immunological neutralization, electron-microscopic appearances and for their leukæmogenic properties in animals.

There have been a considerable number of reports of virus-like particles seen in the blood and cells of leukæmic patients (Anderson, 1965; Dmochowski, 1965). Some of these particles are far too thin to be considered as possessing a viral nucleoid. Others have a more finite structure, either round or with tails. These particles can not be said to be viruses on morphological grounds alone, for the products of cytolysis as well as mycoplasmas closely resemble them. Indeed, identification of known viruses by the electron microscope depends on seeing a large number of particles so that their characteristics can be fully assessed. Animal experiments have shown that oncogenic

(tumour forming) and non-oncogenic viruses have very similar appearances and they cannot be distinguished by electron microscopy alone; the Shope fibroma virus, for example, resembles herpes virus, and these can only be distinguished for certain by biological testing (de Harven, 1965). So far the injection of human leukæmic products—cells and extracts of serum—has failed to induce leukæmia in experimental animals, even when primates are used.

These reports of “virus particles” even if they indicate genuine viruses do not prove that they are the ætiological agents in leukæmia. Some workers consider that even if human leukæmia is of viral origin the viral content may be very low and difficult to demonstrate.

Recently two approaches to the problem of viral ætiology of leukæmia have given most interesting results. The first is the work of Negroni (1964) who isolated an infective agent from the bone marrow in human leukæmia. This agent caused cytopathic changes in human embryonic cell cultures and these cytopathic effects could be prevented by antibodies present in the serum of leukæmic patients. At first this agent was thought to be a virus but later it was demonstrated that a Pleuropneumonia-like organism (PPLo), a mycoplasma, was present in Negroni's material (Grist & Fallon, 1964). However, in spite of this remarkable association with leukæmia, there is no proof that the agent plays any part in the ætiology. The association of mycoplasmas with leukæmia has been confirmed (Girardi *et al.*, 1965) and they have also been found in the lymphoid tissues of patients with reticuloses. The findings are in keeping with the concept that there may be “passenger” viruses and mycoplasmas in leukæmic cells.

Encouraging results have come from the study of the epidemiology of Burkitt's tumour (see page 328). Burkitt's tumour (Burkitt, 1958) is a malignant tumour of lymphoid-like cells that mainly affects the jaws of children. The disease has its peak incidence in equatorial Africa; apart from this endemic zone a high incidence of the disease has been observed in New Guinea and isolated cases have been reported from temperate climates. In tropical Africa the incidence of the disease shows a distinct relation to altitude and rainfall. These climatic factors strongly support the view that the disease could be due to an agent, possibly a virus, spread by an insect vector (Harris, 1965). There is a strong correlation between the geographical distribution of Burkitt's tumour and certain diseases that are well established as being insect borne such as malaria. Several viruses have been isolated from Burkitt's tumour. Epstein, Barr & Achong (1965) and Stewart and co-workers (1965) have observed a herpes-like virus in tissue cultures of cells derived from Burkitt's tumours, but so far this virus has not been demonstrated to be oncogenic; a similar virus has been detected in other cell cultures of human leukæmic cells (Moore *et al.*, 1966). An identified herpes virus (herpes hominis) (Simons & Ross, 1963) has been observed in fresh biopsies of the tumours, but this virus is a common inhabitant of the throats of African children. Bell and co-workers (1964) reported the isolation of reovirus type III from Burkitt's tumour and Stanley (1966) has produced a lymphoid neoplasm in mice by injecting them with reovirus but although the tumour has some superficial resemblance to the Burkitt tumour it can hardly be acceptable as evidence that reovirus is the ætiological agent for the Burkitt tumour.

It is possible that many of the viruses isolated are passengers in the tumour tissue and not ætiologically significant.

Mycoplasmas are an important group of micro-organisms that must be investigated for possible oncogenic properties, either alone or in association with a virus. Mycoplasmas are the smallest micro-organisms which contain the genetic mechanisms and enzyme systems for a free living existence. A number of well characterized mycoplasmas occur in the throat and urogenital tract of man but only one has been unequivocally demonstrated to be a pathogenic agent for man, *M. pneumoniae*, which is the ætiological agent for cold agglutinin positive atypical pneumonia (Chanock, Hayflick & Barlie, 1962). Mycoplasmas are a potential source of confusion to virologists attempting to demonstrate viruses in human leukæmia, as many of their properties are similar. In the electron microscope, mycoplasma particles may be mistaken for viruses and they are common contaminants of tissue cultures employed for the isolation of viruses (Harris, 1965). The techniques for working with these organisms are not as yet entirely satisfactory, but it is likely that knowledge of their biological functions in many will increase as various technical problems in handling and isolating them are solved (Butler & Leach, 1966). Mycoplasmas may possibly be a "secondary invader" of neoplastic tissue (Barlie, 1965).

It is difficult to predict what will come from the large-scale hunt for virus in leukæmia that has been commenced in the United States but in following this research it must be borne in mind that what is small and circular is not necessarily a virus, and even if it is a virus it may not be pathogenic for leukæmia.

### Chromosomes and Cytogenetics in Leukæmia

The analysis of the chromosome number and the karyotype of human chromosomes is in the main dependent on three techniques for obtaining cells in metaphase:

1. *Direct Examination of Tissues having a High Mitotic Index*, i.e. bone marrow and in some diseases lymph nodes and peripheral blood, using a brief period of incubation and colchicine to accumulate metaphases.

2. *Culture of the Peripheral Blood with Phytohemagglutinin*, which stimulates the small lymphocytes to transform into enlarged dividing cells; these cultures are usually examined 48–72 hrs after incubation.

3. *Culture of Cells for examination of the Somatic Karyotypes*. Cells apart from hæmatopoietic tissues can be obtained for analysis by using long term fibroblast cultures, skin and fascia lata being the source of the cells.

In the case of leukæmia, many of the cells in the peripheral blood can divide without phytohemagglutinin. Short-term cultures of such cells can be harvested at 24, 48 and 72 hrs.

It is clear that each of the first two methods for the study of hæmatopoietic tissues in leukæmia provides a different type of dividing cell population. The longer the duration of the culture period the greater is the probability of selection of cells lines which are less representative of the true relative proportion of the different cell lines in life. Furthermore, a very profound alteration in the results obtained is brought about by adding phytohemagglutinin. Such cultures, in the main, will contain dividing lymphocytes

which tend to have normal karyotypes (unless they have been altered by X-rays or drugs), but they will also contain a small proportion of the dividing leukæmic cells that survive. Hence caution must be exercised when assessing the relative frequency of cells bearing a particular chromosome abnormality as it may in part be dependent on the technique used to examine the cells.

Chromosome abnormalities are of various types. First, there may be alterations in the number of chromosomes in the karyotype. These may be simple multiples of the normal diploid number (46), i.e. tetraploid (92), octoploid (184) or they may be of an unusual number, aneuploid. Sometimes the changes in number result in a modal number that is close to the diploid or tetraploid number—these are then referred to as hypo- or hyper-diploid, and hypo- and hyper-tetraploid. If there is no obvious pattern to the frequency of a chromosome number the term aneuploid is used. Secondly, there may be alterations in the structure of chromosomes which may or may not be associated with coincidental changes in the number of chromosomes in the cell.

#### **Acute Leukæmias**

No specific abnormality has been detected in acute leukæmia that is unique to this disease (Hungerford, 1961; Hungerford & Nowell, 1962; Sandberg *et al.*, 1964). In some cases no apparent abnormality of the chromosomes is detectable but in the majority, chromosome abnormalities are present. These abnormalities may involve alterations of the number as well as of the structure of the chromosomes. The abnormalities would appear to be unique to the individual and only carried by the leukæmic cells. In some series of investigations in acute lymphoblastic leukæmia cells were frequently hyperdiploid and in acute myeloblastic leukæmia hypodiploid. The chromosome abnormalities in acute leukæmia are comparable to those encountered in many other malignant tumours. They may arise by a variety of faults occurring during mitosis, such as non-disjunction and chromosome lagging of anaphase. Translocations, deletions, inversions, dicentrics and fragments and other chromosome structural changes may be seen in the leukæmic cells. These aberrations are formed by chromosome breakage and reunion. Occasionally, the failure of the formation of the mitotic spindle results in both sets of replicated chromosomes remaining within a single cell.

#### **Chronic Myeloid Leukæmia**

Chronic myeloid leukæmia is especially interesting from a cytogenetic point of view as in a high proportion of cases there is a specific chromosome abnormality. Nowell & Hungerford (1960) first demonstrated that cells in the bone marrow and blood of patients with chronic myeloid leukæmia have a very small chromosome replacing a normal small acrocentric chromosome. This abnormal chromosome has been named the Philadelphia chromosome, abbreviated to Ph<sup>1</sup>. It is a 21 chromosome with half its long arm missing. It has not been demonstrated in phytohemagglutinin stimulated lymphocytes or skin fibroblasts from chronic myeloid leukæmia patients known to be Ph<sup>1</sup> positive in their bone marrow. In the marrow the Ph<sup>1</sup> chromosome appears to be present in the myeloid, erythroid and megakaryocytic cell lines but not the lymphocytic. Successful therapy and the consequent disappearance of



dividing cells (myeloblasts, promyelocytes, myelocytes) from the peripheral blood, abolishes Ph<sup>1</sup> positive cells in peripheral blood culture. However, examination of the bone marrow in remission clearly demonstrates the Ph<sup>1</sup> chromosome to be present in the dividing cells. The Ph<sup>1</sup> chromosome is unique to chronic myeloid leukæmia, and is not present in other myeloproliferative disorders, i.e. polycythæmia and myelofibrosis even when chronic myeloid leukæmia complicates these diseases. A small proportion of male patients with chronic myeloid leukæmia who are Ph<sup>1</sup> positive have an additional chromosome abnormality, the loss of the Y chromosome in the bone marrow cells (Atkin & Taylor, 1962; Speed & Lawler, 1964). Finally there are a small proportion of patients who have undoubted chronic myeloid leukæmia and remain Ph<sup>1</sup> negative.

The majority of patients with chronic myeloid leukæmia therefore have a specific genetic change in the myelo-erythroid stem cell line that is associated with a change in the karyotype. With blast cell transformation a further series of inconstant chromosome abnormalities appear, resembling those found in some of the acute leukæmias. These, unlike the Ph<sup>1</sup> abnormality, are secondary phenomena. They may be the result of changes in cell growth control and are thought to play an important part in the progression of the disease but not its initiation.

#### Chronic Lymphatic Leukæmia

Gunz, Fitzgerald & Adams (1962) have reported a family living in New Zealand bearing a chromosomal abnormality, deletion of the short arm of one of the 21-22 group. Two members of the family have chronic lymphatic leukæmia and four other members of the family have this chromosomal lesion in their skin cells. Apart from this rarity, no abnormalities have been detected in patients with chronic lymphatic leukæmia. The lymphocytes in this disease are mainly non-dividing cells; measurement of the amount of DNA in their nuclei confirms that they are diploid. Technically these cells are difficult to analyse as the majority of them would appear to be insensitive to phytohemagglutinin, and they cannot be induced to enter cell divisions *in vitro*. During remission phytohemagglutinin can frequently stimulate the remaining lymphocytes, the karyotypes of these cells being normal. This has been used as an argument that there are two populations of small lymphocytes in chronic lymphatic leukæmia, the chronic lymphatic leukæmia cells that cannot respond to phytohemagglutinin and the normal cells which only appear in sufficient numbers to be recognized during remission; the validity of this concept is not conclusive. (Hayhoe, Sinks & Flemans, 1966.)

#### Down's Syndrome and Leukæmia

An interesting line of investigation has been the study of abnormalities of chromosome 21. In Down's syndrome, the patient is trisomic for chromosome 21 (Lejeune, Turpin & Gautier, 1959), and in this disease there is a 20-fold increase in the incidence of acute leukæmia as compared with the general population. Here, the chromosome abnormality is known to antedate the disease. The chronic lymphatic leukæmia cases described by Gunz and co-workers (1962) may be comparable where an abnormality of 21 or 22 predisposes to leukæmia. Chromosome 21 has been thought to be associated

with the gene loci for the control of alkaline phosphatase in the myeloid cells. Chronic myeloid leukæmia patients have low alkaline phosphatase scores (Valentine & Beck, 1951). In mongols with trisomy 21 the alkaline phosphatase is increased, and in the non-leukæmic myeloproliferative disorders (Ph<sup>1</sup> negative) there is no reduction of alkaline phosphatase (Hayhoe, 1960).

Cytogenetic studies alone can produce interesting information about the biological behaviour of particular clones of cells in leukæmia where all the members of the clone have a "marker" by which they can be recognized. Furthermore, in chronic myeloid leukæmia the demonstration of the Ph<sup>1</sup> may have important diagnostic significance. However, it is unlikely that the intensive study of the morphology of the karyotypes *per se* will yield any great advance in our fundamental understanding of leukæmia. The problem that must be faced is what is the nature of the abnormalities of gene function in the leukæmic cells of which disturbance of chromosome morphology may be a visible sign. The answer may come from a closer liaison between the cyto-geneticist and those examining chemical functions at the single cell level.

#### **Chromosomes in Pre-leukæmia**

There are certain hæmatological disorders which have a propensity to terminate as an acute leukæmia. These potential leukæmic syndromes can be grouped into aplastic and myeloproliferative disorders and various atypical disorders which have both aplastic and myeloproliferative features. Rowley, Blaisdell & Jacobson (1966) have reviewed the literature on the chromosome abnormalities in this group of diseases. Many of them have no detectable abnormality of their chromosomes. In three of their own cases and four others previously reported, there were anomalies in the C group of chromosomes (this is the largest group consisting of six pairs of chromosomes). This is an interesting observation as it would appear that the C group is frequently abnormal in acute leukæmia. Rowley and her colleagues have suggested that it is possible that the genes involved in the homeostatic control of the hæmatopoietic tissues may be located on one or more chromosomes in the C group. However, with a few exceptions we are far away from having any knowledge of gene mapping on human chromosomes.

### **TREATMENT OF ACUTE LEUKÆMIA**

#### **L1210 Leukæmia in Mice**

L1210 Leukæmia in mice has been used extensively for chemotherapeutic research. It is a transmissible disease, after a two day lag period cell growth is maintained with a constant replication time of 0.55 days, the cells growing exponentially until the death of the animal. The cells have a high reproductive integrity and the disease can be transmitted by the inoculation of the mouse with a single L1210 cell. It would appear that the death occurs when a critical number of cells have accumulated in the animal; infection and bleeding are not an important feature of this very rapidly fatal disease.

The duration of the life of mice following the injection of the L1210 cells is directly dependent on the number of cells given. There is very little variation in the survival time of a group of animals given the same number of cells. This experimental system has provided useful information on the mode of

action of drugs on the proliferating cells and on the optimum combinations of dose and time of dose (Skipper, 1965).

First, it appears that the percentage of leukæmic cells killed (the fractional kill) with a given dose of a chemotherapeutic agent is constant, regardless of the size of the cell inoculum. Intermittent high doses are more effective than repeated smaller doses. Thus the absolute number of leukæmic cells present in the body influences the chances of eliminating these cells. Secondly, to cure the mouse of L1210 leukæmia all the leukæmic cells have to be killed. These observations have given rise to the cell kill programme of chemotherapy for the treatment of acute leukæmia. On the other hand, study of this model fails to shed any light on some of the more fundamental problems of acute leukæmia in man. Several experimental tumours in rats and mice can be cured by chemotherapy, the results being reproducible. Despite this ability to cure animals of induced neoplasms, undoubted chemotherapeutic cures in human cancer have only been achieved in chorioncarcinoma in women and may have possibly been effected in some cases of Burkitt tumour.

The L1210 leukæmia model differs from acute leukæmia in many respects. The very short generation time of 11 hrs (time between two consecutive divisions), the uniformity of growth, a high growth fraction (percentage of cells that are dividing actively) and the stability of its chromosome number are all expressions of a highly selected population of cells that have a very low mutation rate. In an actively proliferating population of cells such as L1210 leukæmia, the interphase cells pass successively through three stages between mitoses. These stages are related to the state of the DNA in the nucleus.  $G_1$  is a stage immediately after division lasting until the onset of DNA synthesis, S the period of DNA synthesis during which time the genetic information on the chromosome is replicated and  $G_2$  a period after completing DNA synthesis prior to the onset of prophase.

In acute leukæmia in man relatively few cells are found to be in S and  $G_2$ ; the majority appear to be in  $G_1$ . But, unlike the L1210 model it is likely that many of these  $G_1$  cells are in fact not actively engaged in cell division, and may spend considerable periods of time in this state before continuing with division. In acute leukæmia in man it would appear that the blast cells are not uniform. Active division seems to occur mainly in the larger cells. The smaller cells do not synthesize DNA and many of them are probably non-divisional. The DNA contents of these non-dividing cells indicate that they are in the post mitotic pre DNA synthetic phase of their cycle. Estimates of the generation time (time to complete a division cycle) for dividing cells range from 15–20 hrs in children (Mauer & Fisher, 1966) to two–three days in adults (Killman *et al.*, 1963). It is not known what is the lifespan of the leukæmic blast cell, or whether the small cells can revert from their non-dividing state and recommence cell growth and division. Clarkson & co-workers (1965) have shown that continuous infusion of  $^3\text{H}$ -thymidine caused 82 to 93 per cent of the leukæmic cells in the marrow to be labelled, which is a good indication that the majority of these cells had either divided or were preparing for division during this time. Cell division by leukæmia cells is generally less active in the peripheral blood than in the bone marrow. Hence, the  $^3\text{H}$ -thymidine labelling index of peripheral blood, which is a simple procedure, fails to mirror the extent of leukæmic cell proliferation that is

taking place in the blood, and for this reason has only limited value in assessing the progress of the disease. The factors that determine whether a cell remains in or out of division cycle are not known. As yet there is no clear evidence in acute leukæmia in man whether some of the blasts completely lose their capacity for division or whether they maintain a latent ability for division though it may remain suppressed for long periods of time. There is therefore a very fundamental difference in the kinetics of the L1210 model and the growth pattern of human acute leukæmia.

### **Acute Leukæmia in Man**

In man, a classical accompaniment of acute leukæmia is depression of bone marrow function: platelets are profoundly reduced and later the myeloid and erythroid elements are depressed. The simple concept that there is a crowding out of the bone marrow with leukæmic cells leaving no room for other elements is probably incorrect. There is often a great deal of potential space in which the normal marrow cells could proliferate. The marrow depression is more likely to be the outcome of either substrate competition for essential metabolites between the normal and the leukæmic cells, or a form of humoral inhibition of the normal marrow cells by the leukæmic cells. It is well recognized that once a remission is established the bone marrow rapidly recovers its function, and will replenish the circulating elements in the blood even though the patient is still taking cyto-toxic drugs. This aspect of the leukæmia problem, the interaction of the leukæmic cells and the normal cells is of fundamental importance in human leukæmia, but would appear to be almost irrelevant in the L1210 system.

For practical purposes the treatment of acute leukæmia can be considered under two headings, supportive therapy and specific therapy.

### **Supportive Therapy**

#### **Hæmorrhage and Platelet Transfusion**

Anæmia due to aplasia or hæmorrhage often leads to death within a few days of the diagnosis being established. As successful specific therapy may take up to six or even eight weeks before a useful effect is observed these patients must be kept alive by ample blood transfusions in the meantime. However, at any time in the aplastic phase when there is marked thrombocytopenia which may be accentuated temporarily by treatment, hæmorrhage may lead to death by its severity or cause death by occurring in vital organs such as the brain.

The maintenance of hæmostasis by platelets appears to be the result of three functions. The platelets support the endothelial lining of the capillaries, they can form hæmostatic plugs, and can release lipoproteins (factor 3) which play a vital role in blood coagulation (Johnson, Van Horn & Pederson, 1966). There is no precise relation between the platelet count and the incidence of bleeding (Cronkite, 1966), but Gaydos, Freireich & Mandel (1962) who studied this relation in leukæmic patients observed hæmorrhage on 16 per cent of the days when the platelet counts were between 20,000 and 50,000 per cu mm on 92 per cent of the days when the count was below 1,000 per cu mm.

The introduction of a closed system of plastic bags has now made it a practical procedure to collect large numbers of platelets (see Djerassi, 1966 for a discussion of technical aspects of platelet transfusion). When platelet rich plasma (PRP) is transfused within six hours of its collection, about 30 per cent of the injected platelets can be found in the circulation one hour after the transfusion. One unit of PRP (obtained from 500 ml whole blood) contains  $1 \times 10^{11}$  platelets. It is estimated that two units of PRP will give an increment of 25,000–30,000 platelets per cu mm in a 30 kg child. To combat hæmorrhage, the transfusions are needed twice a week during periods of thrombocytopenia. If the patient is febrile or develops anti-platelet antibodies, these injections may be required more frequently (Freireich, 1966). It has been found in practice that a platelet count kept above 20,000 per cu mm considerably reduces the risk of hæmorrhage. The large requirements of fresh blood (70 kg man requires four–eight units twice a week) and the technical difficulty of platelet preparation have limited the use of this treatment in leukæmia. In those centres in the United States where platelet transfusions have been used frequently, there is no doubt of their efficacy in controlling hæmorrhage. The transfusion of platelets is not without risk; fever and allergic manifestations, isoimmunization and very occasionally serum hepatitis may follow. Platelet typing is not practical but care should be taken to obtain donors whose red blood cells are compatible with those of the patient. Ideally, the transfusions should be made as soon as possible after taking the donor's blood; 24 hrs storage virtually renders the platelets useless.

### Infection and Granulocyte Transfusions

A lack of effective granulocytes in all forms of acute leukæmia predisposes to infection. The disease may present with an infective complication or such may appear during its course.

Facilities to protect patients from the general hospital environment are urgently needed. It is hoped that when "reversed isolation" units are available in this country infection will become less of a problem.

The prolonged use of antibiotics prophylactically is so often complicated by superinfection, particularly with monilia, that they should be used only when an infective complication is recognized and nystatin should be given at the same time. The high fever which is a feature of many cases of acute leukæmia does not of itself indicate an infective complication.

Freireich and his colleagues (Freireich *et al.*, 1964; Frei *et al.*, 1965) have had considerable experience of the use of transfusions of granulocytes in the treatment of agranulocytosis in leukæmia. They have shown that infusion of  $10^{11}$  cells/sq meter of the body surface will raise the recipients blood count 1,000 cells/cu mm. Transfusions of  $10^{10}$  or less cells/sq meter have no effect. To provide this large number of leucocytes the only practical procedure is to use the peripheral blood of patients with chronic myeloid leukæmia whose granulocytes retain their phagocytic properties. These donors should not be in the blast phase and should have leucocyte counts above 100,000 cu mm. The leucocytes are obtained by leucophoresis and the same donor can be used several times giving one or two units of blood at a time. Separation of the cells can be improved by dextran or fibrinogen precipitation of the erythrocytes

and gentle centrifugation, but these substances are potentially dangerous if they are repeatedly infused into the donor.

The transfusion of the agranulocytic patient with the chronic myeloid leukæmia cells is frequently followed by a rapid improvement in the patient's condition. Fever declines and infections previously resistant to antibiotics can be controlled. Most notable has been the improvement in patients with *Pseudomonas* septicæmia, which in the absence of the transfusions is usually fatal. Following transfusion, leucocytes rapidly disappear from the blood so that only about five per cent of the number transfused can be recovered one hour later in the peripheral blood. This number of cells then declines exponentially with a half time of about 24 hours. The rapid clearance of the blood is mainly due to the movement of the cells into the depleted granulocyte stores. A patient may require leucocyte transfusions every three-four days for two-three weeks to provide an adequate replacement of granulocytes. Though the cells leave the blood they can be recovered in the bone marrow for several weeks after transfusion—the finding of the Ph<sup>1</sup> chromosome in marrow preparations is proof of the presence of chronic myeloid leukæmia cells and indicates they are capable of forming a temporary graft in the acute leukæmic host. At the concentrations of cells up to  $10^{11}$ /sq. meter no graft versus host reactions have been observed. Schwartzenberg and co-workers (1966) using higher concentrations of leucocytes in their transfusions observed mild graft versus host reactions.

### **Hyperuricæmia**

The vast nucleic acid catabolism that accompanies the successful treatment of acute leukæmia may lead to hyperuricæmia and renal failure. Vogler and co-workers (1966) have reported the use of the xanthine oxidase inhibitor 4-hydroxypyrazole pyrimidine (Allopurinol) in the management of this complication. This drug also inhibits the catabolism of 6-mercaptopurine which may have a possible therapeutic advantage.

### **The Specific Therapy of Acute Leukæmia**

Glucocorticoids and certain cytotoxic drugs have become established in the treatment of leukæmia, but whereas, in children particularly, prolonged remissions are frequently obtained, drug treatment has very rarely appeared to eradicate the disease. The reasons for this are possibly three-fold, first that the treatment necessary to eradicate the disease is lethal, secondly the tumour acquires resistance to the treatment and thirdly there are foci of disease that escape the effects of the treatment.

If treatment controls the leukæmic process an aplastic marrow rapidly regenerates, but if cytotoxic drugs do not induce remission they probably increase the aplasia. The risk of myelotoxicity must however be taken and severe thrombocytopenia and leucopenia are not contraindications to treatment. In an attempt to obtain a remission large doses of toxic drugs may be used such as would not be tolerated for the long term control of the disease. Modern treatment tends to consist of a remission inducing regime followed by a remission maintaining regime. There are few facts on which to base the prognosis as to response and this may be delayed for up to six or eight weeks during which the continuing aplasia demands vigorous supportive treatment.

Hæmorrhagic manifestations, a high peripheral white cell count and according to Boggs, Wintrobe & Cartwright (1962) bone tenderness adversely affect the early prognosis.

Myelotoxicity is the most obvious side effect of cytotoxic therapy and the most serious, but other renewal tissues, particularly the gastrointestinal tract mucosa may be damaged. The early detection of ulcers in the mouth may serve as a warning of impending serious myelotoxicity. Ulcerative lesions in the lower gastrointestinal tract may contribute to what is often a fatal hæmorrhage. Individual cytotoxic agents vary in their side-effects, which will be indicated later, and their myelotoxicity varies but this is to a large degree common to them all. In spite of the hazards of treatment side-effects do not appear to be the factor that limits the success of the treatment of acute leukæmia in man.

Clinical experience suggests that the development of resistance by the tumour is a limiting factor. If an intermittent therapeutic scheme is used and treatment withheld during remission, it is frequently the case that a drug that was successful in inducing the primary remission is ineffective in a relapse, but that an alternative drug is effective. If therapy is continued during remission, inevitably sooner or later the leukæmia escapes the control of that drug but may respond to an alternative.

Of great importance when considering the limitations of leukæmic therapy is the failure to irradiate certain foci of leukæmic growth. The disease can frequently be eradicated from the peripheral blood and less frequently from the bone marrow but nevertheless it almost always returns. This could be due to the persistent operation of leukæmogenic factors, but the persistence of the disease elsewhere is an undoubted factor.

Mathé and co-workers (1966) have conducted a series of extensive biopsies on 31 patients in apparent complete remission. Twelve out of 31 had evidence of leukæmic blast cells in bone marrow, liver, kidney, testicle and C.N.S.; further courses of chemotherapy reduced the incidence of these hidden cells. Nies, Bodley & Thomas (1965) made post mortem examination on 15 patients with acute leukæmia in remission who had died from causes other than leukæmia. In ten of these patients there was microscopical evidence of leukæmic cells in the liver, kidney, C.N.S. and lungs. The frequency and importance of involvement of the C.N.S. has, perhaps, not been widely appreciated. Neurological manifestations may be present even though the patient is under good hæmatological control (Hyman *et al.*, 1965). Leukæmic meningeal infiltration presents with signs of increased CSF pressure and vomiting, headache and papillœdema are frequently observed. The VII<sup>th</sup> nerve is the most frequent cranial nerve to be involved but any may be affected.

### Anti-leukæmia Agents

Only those anti-leukæmic agents in current general use will be discussed.

**Glucocorticoids** have an established place in the treatment of acute leukæmia in man. They are most effective in the treatment of acute lymphoblastic leukæmia, especially in children in whom Boggs, Wintrobe & Cartwright (1962) observed clinical and hæmatological improvement in 91 per cent. The equivalent figure in adults was 63 per cent. The Medical Research Council's Working Party (1963) reported a remission rate of 42 per cent in lymphoblastic

leukæmia in all age groups. The MRC study also indicated that the median survival time in all forms of acute leukæmia was reduced by high steroid dosage (prednisolone 250 mg daily) compared with orthodox dosage (40 mg) and even with those given no steroids. All these patients received 6-mercaptopurine (6-MP).

There is less agreement as to the value of glucocorticoid therapy in acute myeloblastic leukæmia. Boggs and co-workers (1962) stated that 98 per cent of such cases did not benefit and suggested that the drug may be harmful in this condition. On the other hand, Roath, Israëls & Wilkinson (1964) observed a hæmatological remission in 13 of 83 cases of acute myeloblastic leukæmia treated with glucocorticoids alone. A further MRC trial (1966) confirms their experience and indicates that prednisolone alone in a dose of 40 mg daily is at least as effective as 6-MP alone.

Glucocorticoids should be used principally as remission inducing drugs but they may have value apart from this as part of supportive therapy. The intense malaise, fever and the bone pains of both acute lymphoblastic leukæmia and acute myeloblastic leukæmia may be rapidly improved by treatment and there exists a general impression that they reduce the tendency to hæmorrhage.

**6-Mercaptopurine (6-MP)** was introduced into the treatment of acute leukæmia by Hitchings & Rhoads in 1954. 6-MP has its main application in the induction and maintenance of remission in acute myeloblastic leukæmia in adults, but should be used in acute lymphoblastic leukæmia in both adults and children when the disease escapes control by other drugs. It is severely myelotoxic, and may cause nausea and vomiting, and it is also hepatotoxic. The dose for remission induction is 2.5 mg/kg body weight/day and this dosage should be maintained for not less than eight weeks before concluding that the disease will not respond. If a response is obtained then the dose should be halved, but raised again if the disease escapes control. The drug is worth trying in acute monoblastic leukæmia but the results are poor.

**Amethopterin (Methotrexate, MTX)**, a folic acid antagonist, has replaced a closely related compound aminopterin in leukæmia therapy. Folic acid antagonists were introduced by Faber and co-workers in 1948. The paramount indication for the use of amethopterin is the maintenance of a steroid induced remission in acute lymphoblastic leukæmia in children. It seems to be less valuable in the acute lymphoblastic leukæmia in adults but should be used when 6-MP fails to control this disease or acute myeloblastic leukæmia. This drug is also extremely myelotoxic and tends to produce buccal ulceration. The daily dose is 2.5 to 5 mg by mouth. It can also be given intravenously. Perrin, Mauer & Sterling (1963) have reported remissions in children after oral therapy had failed with intravenous therapy.

Clinical involvement of the central nervous system is much more common than is realized and can often be effectively controlled with intrathecal amethopterin (Hyman *et al.*, 1965). The drug should be injected in a dose of 0.2 mg/kg body weight on alternate days for eight days. One week later the course should be repeated if the CSF cell or protein content is increased or the pressure raised. Hyman *et al.* (1965) reported that 57 out of 67 episodes of CNS involvement responded to chemotherapy. If treated CNS episodes did not appear to have any significant effect on prognosis but their recurrence



rate is high. If the patient fails to respond to intrathecal amethopterin, radiation of the skull may be valuable (Galton, 1966b).

**Vincristin**, an alkaloid of periwinkle (*Vinca Rosea*, Lynn), was isolated by Noble, Berr & Cutts in 1958. Its anti-leukæmic properties were demonstrated by Karon, Freireich & Fry in 1962. Vincristin is the most powerful anti-leukæmic agent so far discovered. Johnson *et al.* (1963) reported complete remissions in 40 per cent and partial remissions in a further 30 per cent of children under ten many of whom had relapsed on other treatments. If used initially the remission rate approaches 90 per cent. The drug is very toxic, but fortunately less so in children than in adults. Its myelotoxicity seems to be less than that of 6-MP or amethopterin, however it is intensely neurotoxic, affecting the peripheral nervous system and the autonomic nervous system. Peripheral paræthesiæ, anæsthesia and motor weakness frequently complicates the prolonged use. Loss of hair is also a common complication. The drug should therefore be used only as a remission inducing agent and not for maintenance therapy. It should be given only at weekly intervals in a dose of 2 mg/m<sup>2</sup> body surface. It is more effective in acute lymphoblastic leukæmia than in acute myeloblastic leukæmia and is occasionally effective in acute monoblastic leukæmia.

### Treatment Schedules

Until a few years ago it was customary to give interrupted courses of treatment in acute leukæmia, but present practice is to continue treatment during remission.

Cross resistance does not appear to be a problem so that when one drug falls another should be tried. Many schedules are in use for the treatment of acute leukæmia; suggested treatment plans are indicated in Table 10.II.

TABLE 10.II  
SCHEDULES FOR THE TREATMENT OF ACUTE LEUKÆMIA  
IN CHILDREN AND ADULTS

	Type	Remission Induction	Remission Maintenance	1st Relapse	2nd Relapse
CHILDREN	ALI.	Corticosteroids	Amethopterin	Steroids followed by 6-MP	Vincristin
	AML	Corticosteroids or Amethopterin	Amethopterin	6-MP	Vincristin
	ALL	Corticosteroids	6-MP	Amethopterin	Vincristin
ADULTS	AML	Corticosteroids or 6-MP	6-MP	Amethopterin	Vincristin
	A Mono L	6-MP	6-MP	Amethopterin	Vincristin

Recently attempts have been made to obtain improvement in the rate of remission particularly after the failure of orthodox treatment by giving a combination of a number of anti-leukæmic agents. The rationale of this is based on the belief that the drugs used exert their anti-leukæmic effect upon different chemical systems in the cell and that their myelotoxicity is not the sum of the myelotoxicity of each agent: nevertheless the toxicity is extremely high. Various combinations of drugs have been tried but that currently in the widest use is a combination of Vincristin, amethopterin, 6-MP and prednisolone (VAMP) (Frei & Freireich, 1965). It is inadvisable to use this type of treatment in the absence of facilities for prolonged supportive therapy of severe bone marrow aplasia. A typical regime is Vincristin 2 mg/m<sup>2</sup>/week, amethopterin 20 mg/m<sup>2</sup>/every four days i.v., 6-MP 60 mg/m<sup>2</sup>/day and prednisolone 40 mg/m<sup>2</sup>/day. The drugs are given until a remission is obtained, when treatment is withheld for ten days. Five ten-day courses are then given at ten-day intervals and after this no further therapy is given until the patient relapses when the course may be repeated.

### Results

It is not easy to assess the effect of cytotoxic drugs on the course of acute leukæmia, for their introduction has coincided with improved supportive therapy. Nevertheless there is considerable evidence that the median survival time has been increased by their use.

Tivey (1954) reported data collected from the literature of 570 untreated cases of acute leukæmia in children and found a median survival time from diagnosis to death of 2·4 months, and of 1·7 months in 172 adults. Two large series of treated cases indicate a considerable increase in the survival time. Roath, Israels & Wilkinson (1964) reported a median survival time of six months in acute lymphoblastic leukæmia and 3·5 months in acute myeloblastic leukæmia. Over a prolonged period of study they noted a considerable improvement coinciding with the introduction of corticosteroids and cytotoxic drugs. Boggs, Wintrobe & Cartwright (1962) recorded a survival time of seven months in acute lymphoblastic leukæmia in children and indicated that amongst their recent cases there was a further improvement. Studies on material from the Memorial Hospital, New York for the period 1926–1947 showed that only three patients in a series of 150 survived for more than one year and all of them were dead at 14 months (Southam *et al.*, 1951). The impact of chemotherapy is clearly seen in a further analysis of the survivals at the Memorial Hospital: the median survival time for children with acute leukæmia in 1948–49 was five months but had risen to 13 months in 1959–63. The ten per cent survival had risen from six months to 26 months during the same period, but only a few survived to five years (Burchenal, 1965). Burchenal (1965) conducted a world wide survey to obtain evidence of long term survival in acute leukæmia; 20 adults and 75 children were reported to have survived longer than five years.

Remission is the main factor in determining the survival time of the patient. The probability of inducing a remission in a patient under the age of 20 with acute leukæmia has been estimated to be as high as 90 per cent (Freireich, 1961).

Sixteen children with acute leukæmia have been given VAMP (Frei & Freireich, 1965). Thirteen obtained remission in the first course, 11 completed the full therapy and two have been reported to be in complete remission 650 days after ending the course of therapy. No real conclusion can be drawn from such a small series, a two-year remission in two out of 16 is a promising response.

### **Bone Marrow Grafting**

Studies in rats and mice have shown that death following a lethal dose of total body irradiation can be prevented by giving the irradiated animal a graft of isologous bone marrow. The injected bone marrow cells are responsible for the repopulation of the hæmatopoietic and lymphoid tissues. Whole body irradiation has subsequently been used in the treatment of leukæmia and other malignancies in man. Patients are given a lethal dose of whole body radiation followed by an injection of allogeneic bone marrow. Unfortunately this treatment which works so well in mice is a failure in man. Mathé and his group in Paris (1965) gave 13 patients 750–850 rads total body radiation followed by a bone marrow graft. Four died due to a reaction to the graft, five died as the result of a severe secondary syndrome and one died from chickenpox, after the graft had taken. Two had transient grafts and one survived 20 months finally dying of a viral infection; no evidence of leukæmia was present at the time of his death.

The main immediate hazard that accompanies the grafting of allogeneic hæmatopoietic cells is the secondary syndrome. This syndrome is due to an intense graft versus host reaction. The allogeneic immunologically competent cells in the graft recognize the host as foreign and react against the host's tissues. The pre-irradiation of the host prevents the host reacting against the graft but not the graft versus host reaction. Fever, emaciation, vomiting and diarrhœa accompanied by hepatomegaly and a desquamative dermatitis are the main features of the syndrome. Aplasia of the intestinal crypts and of the lymphoid tissue constitute part of the pathological change. The timing and the severity of the secondary syndrome has been found to be quite different in man compared with rats and mice. In man it is early and severe. In rats and mice the disease occurs later and in a much milder form, which can be modified and controlled by treatment. There are two additional reasons for the failure of this form of cell grafting in man, first the difficulty of collecting a sufficiently large quantity of human bone marrow and secondly the decrease in its restorative capacity following storage. At present bone marrow transplantation in acute leukæmia has been largely abandoned. This is particularly disappointing as in mice it has been demonstrated that a controlled secondary disease can destroy leukæmic cells which survive after a high dose of total body irradiation. It has been shown that in other primates the reactions of the bone marrow are more closely allied to those seen in man and perhaps a study of these animals may give clues as to the ideal management of allogeneic bone marrow grafts.

Schwarzenberg and co-workers (1966) have attempted to obtain a remission in acute leukæmia patients by transfusing them with leukocytes taken from patients with chronic myeloid leukæmia. They observed that when sufficiently large numbers of these cells are transfused into acute leukæmic

patients, whose bone marrow had been depressed by chemotherapy, a mild form of secondary syndrome occurred, which may be accompanied by a remission. However, the effects of this procedure were transient; at the best, the remissions lasted only a few weeks.

This immunological approach to the treatment of leukæmia, mediated through the action of foreign immunologically competent cells, has several features which are attractive to the biological theorist, but as yet the application of the principle to man is not practical.

### ( CHRONIC MYELOID LEUKÆMIA (CML)

It is helpful first to consider normal myelopoiesis before seeing how this differs in chronic myeloid leukæmia. During the past ten years considerable advance has been made in the study of myelopoiesis, mainly due to the use of radioactive markers to study the fate of cohorts of cells in the marrow or in the blood. The cells that make up the myelopoietic tissue can be divided into two functional groups. The proliferating cells are stem cells, myeloblasts, promyelocytes and myelocytes. The non-dividing maturing cells are meta-myelocytes, band forms and mature granulocytes. The precise details of the proliferation kinetics of the first group in man are still uncertain. Current estimates suggest that it takes about six days for a cell to pass through the proliferation series, that is from the stem cell to the last myelocytic division. There then follows a period of three-four days during which time the cell matures finally ending with its release into the circulation as a mature granulocyte. Studies of granulocytes labelled with radioactive diisopropyl-fluorophosphate ( $DF^{32}P$ ) have shown that normal granulocytes leave the circulation in an exponential fashion with a half-life of six-eight hours (Athens *et al.*, 1961). This means approximately 1/10th of the circulating granulocytes are replaced each hour. The granulocytes leave the blood by a random loss through epithelial surfaces (Cronkite, 1964). It is likely that the main site of loss of the cells is the lungs, in which the epithelial surface area is extremely large; the gastro-intestinal tract is the other principal site of loss. The granulocytes in the blood are in two states, circulating and marginated. The circulating cells are moving with the blood stream, the marginated cells are attached to the blood vessel walls. These two groups of cells are in equilibrium, being approximately equal in number, and they change rapidly from one state to the other. The mature granulocyte when it leaves the bone marrow does not return to the marrow. Cell death is the fate of all granulocytes for unlike small lymphocytes they lack any potential for division (Craddock, 1965; Cartwright *et al.*, 1965).

In chronic myeloid leukæmia there is a gross over expansion of the granulopoietic tissues, so that cell production is active in the bone marrow, spleen, peripheral blood and various other tissues that are infiltrated by chronic myeloid leukæmia cells. The cell production pathway is the same as in normal myelopoiesis, though differences of enzyme content (i.e. low alkaline phosphatase) and the  $Ph^1$  chromosome are indications that the chronic myeloid leukæmia cells are not in all aspects identical to normal myeloid cells. The chronic myeloid leukæmia granulocytes leave the circulation at the same rate as normal granulocytes and they have similar phagocytic and anti-bacterial responses. Unlike the mature granulocyte the immature cells

in the blood in chronic myeloid leukæmia do not possess phagocytic and anti-bacterial properties and they recirculate from the blood to the marrow and spleen. The overall production rate of mature granulocytes in terms of the numbers of immature cells present would appear to be close to that seen in normal myelopoiesis; however, there is evidence to suggest that the rates of cell division may differ in the spleen, bone marrow and peripheral blood. Myelopoiesis in normal bone marrow is under a homeostatic control so that production and demand are in balance. There are many possible factors operative in this control process which will not be discussed here. In chronic myeloid leukæmia the myelopoietic cells would appear to be insensitive to the influences of the normal homeostatic controls, so that a progressive expansion of the myeloproliferative tissue mass occurs, which is completely unrelated to any demand for the end product.

Chronic myeloid leukæmia is the rarest form of leukæmia. It may appear *de novo*, or constitute the final stage of other myeloproliferative diseases, such as polycythæmia rubra vera and myelofibrosis. The development of chronic myeloid leukæmia, as well as acute myeloblastic leukæmia, in polycythæmia rubra vera is probably influenced by the use of  $^{32}\text{P}$  therapy.

In polycythæmia rubra vera the white cell count is usually raised and the leukæmic change is insidious and may be difficult to determine; of great value is the estimation of the granulocyte alkaline phosphatase which falls to leukæmic levels. The  $\text{Ph}^1$  chromosome is not found. Leukæmic change in myelosclerosis is less common but the pattern of the alkaline phosphatase and the  $\text{Ph}^1$  chromosome is similar.

Chronic myeloid leukæmia is predominantly a disease of middle and old age, but it is occasionally seen in children. Hardisty, Speed & Till (1964) describe two forms of chronic myeloid leukæmia in children, a type which resembles the disease seen in adults and a juvenile type in which lymph node enlargement is greater and splenomegaly less than in the adult type. In both forms the leucocyte alkaline phosphatase is low, but only the adult type is  $\text{Ph}^1$  positive. Reviewing the literature they found an incidence of 26 juvenile and 37 adult types of chronic myeloid leukæmia in 1,119 children with leukæmia.

### Treatment

Infection and hæmorrhage are not common features of chronic myeloid leukæmia, but the high platelet and perhaps white cell content of the blood predispose to thrombosis. Supportive measures are not usually required therefore during the period that specific treatment is being applied, excepting that anæmia may sometimes need to be corrected by transfusion. It is the most satisfactory of the leukæmias to treat because of the very high response rate to treatment. Treatment was revolutionized by the introduction of busulphan (Myleran) by Galton (1953). Until then radiotherapy to the spleen had been successfully used, but the course of the disease was one of relapse, treatment, relapse, etc. with a less satisfactory and shorter response to each course of radiotherapy.

Busulphan, once a remission has been induced, can be continued indefinitely in doses that are not myelotoxic yet sufficient to suppress the leu-

kæmia. In the doses required to produce the initial remission (up to 12 mg daily) marked aplasia may occur but usually recovers.

It is paradoxical that whereas a higher response rate and more satisfactory control can be obtained with busulphan in chronic myeloid leukæmia than with drugs in other forms of leukæmia yet the median survival time has not been increased by their use. Bodley Scott (1957) compared the survival of a group of treated cases of chronic myeloid leukæmia with a group of untreated cases reported by Minot, Buckman & Isaacs in 1924. There was no significant difference. The reason for this is that whereas busulphan produces near normal health in remission, acute myeloblastic change which so often leads to the fatal termination of chronic myeloid leukæmia occurs with equal frequency in treated and untreated cases.

Prolonged busulphan therapy may be complicated by interstitial pulmonary fibrosis (Oliver *et al.*, 1961). A syndrome consisting of pigmentation, anorexia and weight loss clinically suggesting Addison's disease and also cataract are rare complications.

### CHRONIC LYMPHATIC LEUKÆMIA (CLL)

Chronic lymphatic leukæmia has its maximum death rate in the United Kingdom at about 75 years of age (approximately 130 per 1,000,000 men at risk). There is a linear increase from about 45 years of age (less than 10 per 1,000,000 men at risk) to 75. The sex ratio for men and women is 2 : 1. It is interesting that in certain countries, Japan in particular, and probably in Malaya and India, chronic lymphatic leukæmia is extremely rare. This is suggestive of a possible genetic link in the susceptibility to the stimulus for leukæmogenesis (Doll, 1965).

During the past ten years there has been a great advance in the understanding of the functions of lymphocytes and of the inter-relation of the various lymphoid tissues in the body. Lymphocytes are now known to play an important role in immunological reactions both as a step in the production of humoral antibody, in histo-incompatibility reactions and in delayed hypersensitivity. Previous notions that the small lymphocytes were cells of short life span have been found to be incorrect. The majority of the small lymphocytes in man probably survive several months and a few may persist for a few years. As would be expected from their long life span, the daily production rate is low. Gowans & Knight (1963-64) have demonstrated that in rats there is a continuous recirculation of lymphocytes from the blood to the lymphatic system and back to the blood.

The small lymphocyte is the end product of lymphocytopoiesis and so long as it retains the morphological characteristics of the small lymphocyte it does not divide. However, when the cell is exposed to an appropriate stimulus either an antigen to which it is sensitive or a non-specific stimulus, such as phytohemagglutinin *in vitro*, cell growth and cell division are initiated. Experiments have demonstrated that small lymphocytes possess the genetic information to undergo all the changes that are a part of the cell transformation reaction, but normally these genes are repressed. The recognition of an appropriate stimulus by the small lymphocyte leads to a de-repression of the genes controlling cell growth and division (Cooper & Amiel, 1965).

In chronic lymphatic leukæmia the daily production rate of lymphocytes

is very low and the life span of the cells would appear to be very long. It is possible that they have some biological advantage compared to normal lymphocytes which enables them to survive for a long time in the body. The immunological capacity of the patient with chronic lymphatic leukæmia is frequently reduced, so that their responses to various antigenic stimuli are feeble and there may be depression of the level of circulating immunoglobulins. Many investigators have demonstrated that the chronic lymphatic leukæmia lymphocytes either fail or show a greatly reduced response to stimulation with phytohæmagglutinin *in vitro*. This is an intrinsic defect in the cells and not mediated through a factor in the plasma. This suggests that the chronic lymphatic leukæmia small lymphocyte may have a different form of irreversible gene repression that does not become derepressed on antigenic stimulation. Alternatively, the repression may be normal but the ability to receive and react to antigenic stimuli may be at fault. Whatever the explanation may be, these studies have shown the chronic lymphatic leukæmia lymphocyte to be dissimilar to the normal small lymphocyte. Unfortunately, the investigation of the reason for a cell to fail to respond to a stimulus is often far more difficult than to study the relation between the stimulus and response. At present it is not known whether this reduced immunological reactivity is a factor in the prolongation of the survival of the chronic lymphatic leukæmia lymphocyte. There is no clear evidence as to whether there exists a population of normal lymphocytes which, in the patient with chronic lymphatic leukæmia, are greatly over-shadowed by the excess of pathological lymphocytes. In some instances a restoration of ability to respond to phytohæmagglutinin has been reported to occur when the blood count has been reduced to normal by chemotherapy (Hayhoe, Sinks & Flemans, 1966).

### Clinical Course

Whereas the cases of chronic myeloid leukæmia form a fairly homogeneous group, there is a great variation in the manifestations and natural history of chronic lymphatic leukæmia. A significant proportion of cases are detected on routine blood examination and are symptomless without anæmia. The lymphocyte counts in such cases may be no higher than 20,000 per cu mm. In some cases it may rise for a short while and then remain fairly constant. In others, an initial increase is not observed and they may remain in a fairly steady state for as long as 20 years.

The maintenance of a fairly constant lymphocyte count in the blood of such patients with chronic lymphatic leukæmia could indicate that the production of new cells was under homeostatic control. Alternatively, there may be a difference in the relative numbers of cells in the blood to those infiltrating the tissues in these two groups of cases. In experimental animals there is good evidence that small lymphocytes recirculate from the blood to the lymph nodes and return to the blood via the efferent lymph. Recently, it has been reported that in patients with chronic lymphatic leukæmia, with high lymphocyte counts in the blood, the lymphocyte content of the thoracic duct lymph was surprisingly low (Binet *et al.*, 1966). If this observation reports the true state of affairs in this disease and the low cell counts in the lymph are not due to technical reasons, then this casts some doubt as to whether lymphocyte recirculation is normal in chronic lymphatic leukæmia.

Lymphocyte infiltration of the bone marrow in chronic lymphatic leukæmia is an early event, being found in the majority of those patients found to have chronic lymphatic leukæmia by chance. The effects of the chronic lymphatic leukæmia infiltration of the marrow on the marrow function appears to be variable. In some patients there is a depression of leucopoiesis and erythropoiesis; others with comparable infiltration have normal bone marrow function. There is a striking difference between the relatively benign infiltration in chronic lymphatic leukæmia and that seen in acute leukaemia where bone marrow depression is an invariable. This suggests that it is perhaps not only the numbers of infiltrating cells that depress marrow, but that in some cases they influence bone marrow function in some other manner. Other types of chronic lymphatic leukæmia are progressive; even in some of these cases there may be no evidence of marrow aplasia.

A rapidly rising lymphocyte count and prominent lymphadenopathy are, however, usually associated sooner or later with anæmia and general ill health and the course in such patients may be rapidly downhill. Galton (1966) has recently reviewed 88 cases of chronic lymphatic leukæmia and has come to some conclusions about the evolution of the disease. He considers that there is a continuous spectrum of disease ranging from chronic lymphatic leukæmia to lymphosarcoma. A precise diagnosis can only be reached when the findings in the peripheral blood, bone marrow and lymph node biopsy are considered. In the majority of cases, the diagnosis will be fairly straightforward, but in some patients with histological features common to both chronic lymphatic leukæmia and lymphosarcoma it may be very difficult to classify. This is of more than academic interest, for chronic lymphatic leukæmia and lymphosarcoma have very different prognoses and their management is dissimilar.

### **Immunological Abnormalities in Chronic Lymphatic Leukæmia**

It is now well recognized that a variety of immunological disturbances can occur in patients with chronic lymphoproliferative diseases, especially in chronic lymphatic leukæmia. Autoimmune hæmolytic anæmia, idiopathic thrombocytopenia, rheumatoid arthritis, SLE and Sjögren's syndrome have all been reported in patients with chronic lymphatic leukæmia.

Disturbances of plasma proteins are frequently present in chronic lymphatic leukæmia; of 61 chronic lymphatic leukæmia patients studied by Creyssel and co-workers (1958), 21 had hypogammaglobulinæmia and suffered from recurrent infection. Ultman and co-workers (1959) described 60 patients with chronic lymphatic leukæmia, 19 of whom had hypogammaglobulinæmia; pulmonary infections occurred in 12 and skin infections in 12 of them. In the 22 patients with chronic lymphatic leukæmia investigated by Miller, Budinger & Karnofsky (1962) 11 had hypogammaglobulinæmia and recurrent staphylococcal and pneumococcal infections. Herpes zoster is also seen in chronic lymphatic leukæmia. In chronic lymphatic leukæmia the serum albumin is usually normal; the hypogammaglobulinæmia occurs in both treated and untreated cases (Boggs & Fahey, 1960).

It is interesting to note that tuberculosis has not been reported as a complication of chronic lymphatic leukæmia with hypogammaglobulinæmia, though it is a frequent complication in agammaglobulinæmia. Delayed



hypersensitivity reactions, e.g. to tuberculin, would appear to be little affected (Parkes, 1958).

In the early phases of chronic lymphatic leukæmia when the patients general health is good there is little depression of the cutaneous reaction to a wide range of microbial antigens (Lamb *et al.*, 1962). Later in the disease when the patients general health is poor there is a decline in delayed hypersensitivity reactions. This is in marked contrast to the findings in Hodgkin's disease where cutaneous anergy is present in about 52 per cent of the patients in the early stages of the disease (Lamb *et al.*, 1962).

### Treatment

There is little dispute that chlorambucil (Galton *et al.*, 1955) is the drug of choice in the management of chronic lymphatic leukæmia. Practice, however, varies in the selection of cases requiring treatment. The non-progressive cases should be left untreated and little is lost by observing non-anæmic progressive cases before deciding whether treatment is necessary. Chlorambucil is given in a dose of 10–12 mg daily to induce a remission and the aim should be to reduce the wbc to 10–20,000 per cu mm, when a maintenance dose of 1 to 5 mg a day may suffice to keep it at that level. In some cases, though the leukæmic element of the disease is controlled by chlorambucil, lymphoid masses may persist and particularly if intra-abdominal give rise to considerable discomfort. In such cases a trial of one of the alkaloids of periwinkle, vinblastine or vincristine is worthwhile.

Infection is often a problem and will require suitable antibiotic therapy and occasional transfusion of blood or gamma globulin.

Hæmolytic anæmia is a frequent complication of chronic lymphatic leukæmia. It is usually associated with a positive Coombs' test and once established behaves autonomously and does not vary with the activity of the leukæmia. It does not respond to chlorambucil but is usually controlled with cortico-steroids even if the leukæmia is untreated.

There have been attempts to treat chronic lymphatic leukæmia by extracorporeal irradiation (Lajtha *et al.*, 1962; Thomas *et al.*, 1965) by pumping the patient's blood through a coil surrounding a radioactive source. The patient is screened from the source so that only his blood is irradiated. In this manner high doses can be given to the leucocytes without any of the attendant dangers of local radiation. In one small series (Thomas *et al.*, 1965) doses of 900–27,000 rads were given to the cells by this method, the circulation repeatedly passing through the machine for several hours. Four patients were treated and all of them showed a fall in their lymphocyte count. In one of them the count remained low for nine months but in another it rapidly rose to its former level. The other two cases had not been observed long enough to reach a conclusion as to the long term effects. The method is based on fairly sound radio-biological reasoning. It will certainly cause the death of large numbers of cells and can be used in conjunction with chemotherapy directed at the proliferating elements in the lymphoid tissues. j

### BURKITT'S TUMOUR

In 1958 Burkitt described a clinical syndrome of tumour of the jaw associated with multiple visceral tumours in East African children involving

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FIG. 10.1.

*Left:* Left maxillary tumour before treatment.

*Right:* Appearance 414 days after a single dose of Melphalan (4.5 mg/kg body wt. i.v.).

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mainly the liver, ovaries, adrenals and kidneys. Paraplagia from extra-medullary growths is not uncommon. Involvement of lymph nodes is rare. The histological appearances of the tumours are remarkably uniform and are independent of their site of origin (Wright, 1963). The tumour is comprised of two cellular elements, poorly differentiated lymphoid cells amongst which macrophages are interspersed. There is an apparent discrepancy between the high mitotic index and  $^3\text{H}$ -thymidine labelling index which indicate rapid cell division, and the observed rate of growth of the tumour. It has been suggested that the tumour cells turn over rapidly but the proliferation is matched by a high cell death rate (Cooper, Frank & Wright, 1966). The histiocytes frequently contain remnants of phagocytosed tumour cells, which provides further evidence of a high death rate in the tumour. Unlike carcinomata these tumours are rarely necrotic unless they are massive and secondarily infected. At first it was considered that the Burkitt tumour was the same as childhood lymphosarcoma, but there are now known to be several differences in the natural history of these two diseases which strongly support the idea that Burkitt's tumour is a distinct entity. A notable difference is that the Burkitt tumour rarely has a terminal leukæmic phase, whilst this is frequently encountered in childhood lymphosarcoma.

An outstanding feature of Burkitt's tumour has been the response of the tumour to chemotherapy; prolonged and possibly complete remissions have been obtained (Fig. 10.1). At present, approximately 15 per cent of the children treated in East Africa can be given a prolonged remission (Wright, 1966). Clifford (1966) reported the fate of 57 children with Burkitt's tumour seen in Nairobi between 1963 and 1966. Twelve of them died shortly after admission, four were still under treatment; of the remaining 41, eight had complete remission, 31 had died and two were lost to follow up. In assessing these results it should be borne in mind that many of the children had widespread disease when first seen, and intercurrent infections, malaria and chronic anæmia are very common in these patients.

It has been suggested that tumour regression could be due to a combination of the cell killing effects of the drugs and an immunological response against the tumour by the host. Klein and co-workers (1966) have demonstrated that certain virus induced lymphomas of mice contain specific antigens which induce the formation of specific anti-sera against them. They have also demonstrated that the sera of patients with Burkitt's tumour who have responded to chemotherapy contain antibodies that react with the surface of Burkitt tumour cells. The most reactive sera were obtained from some of the patients in total regression many of whom had been given intensive chemotherapy. The specificity of this reaction is not conclusively established though the evidence so far available suggests that the reaction may well be Burkitt tumour specific.

This line of research is of great importance as there is considerable evidence to indicate that it is a synergistic action of chemotherapy or radiotherapy and the host immunological reaction that is responsible for the cures that can be induced in experimental tumours of animals. It is possible that if Burkitt's tumour possesses a specific antigenicity, ways may be found to enhance this antigenicity and increase the patient's reactivity against the tumour. At present, Burkitt's tumour is the only malignancy of the lymphosarcoma-

leukæmia group in children that has any real chance of being eradicated by chemotherapy. Most success has been achieved by the use of alkylating agents.

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## CHAPTER 11

# TEMPERATURE REGULATION AND ITS DISORDERS

by

K. E. COOPER

OVER the past decade there has been considerable interest in the regulation of body temperature. The clinical problems of greatest interest have been in particular the causes and treatment of hypothermia and the mechanism of fever. While much is now known about the responses of man to considerable changes in his environmental temperature, the central regulating mechanism of this extremely accurate homœostatic process is still clouded in obscurity. This review will *outline* the current concepts of thermoregulation and then deal with abnormal conditions of body temperature such as are met frequently in clinical practice. Obviously a complete presentation of the earlier evidence is impossible, and the reader is referred to four reviews: Atkins (1960), Hardy (1961), Bligh (1966a) and Cooper (1966).

## TEMPERATURE REGULATION

### What is Regulated?

One of the more useful concepts in understanding temperature regulation is the arbitrary theoretical division of the body into "core" and "shell" (Aschoff & Wever, 1958). The core is made up of the intracranial, intrathoracic and intra-abdominal contents, and the shell of trunk skin, subcutaneous tissue and muscle, and the limbs. Precise control is exercised over the core temperature by allowing wide fluctuations in temperature and heat content of the shell. The concept then allows that at any given central temperature there may be considerable fluctuations in the state of *body heat storage*. This initial state of the heat stores can be of considerable importance in determining the outcome of, for example, an acute cold exposure; and the capacity of this heat reservoir is a major factor in determining the large fluctuations of central temperature which can be found in infants. Again, if the lower limbs have been "chilled to the marrow" then exercise, which results in a large flow of blood to their muscles, will result in loss of heat from the core to the muscles; and if this exceeds the metabolic heat production of the muscles, a fall or "after-drop" in core temperature may occur. The core temperature is regulated within narrow limits fluctuating at rest by about 1.1°C (2°F) on a definite diurnal cycle. This temperature is lowest in the early morning and highest in the late evening. The mean level about which the diurnal rhythm swings is to some extent dependent on the environmental conditions (Adam & Ferres, 1954), and is raised in the latter half of the menstrual cycle. Eating, drinking and exercise all induce transient changes in core temperature. The presence of a diurnal rhythm requires an intact



regulating mechanism, the rhythm takes at least five days to adjust to a new time schedule after, for example, flying from Europe to the U.S.A.; the nature and mechanism of the biological "clock" which determines it are not known (Cloudsley-Thompson, 1961).

In clinical practice the core temperature is assessed by measurements made in the mouth or the rectum, the rectal temperature being about  $0.56^{\circ}\text{C}$  ( $1^{\circ}\text{F}$ ) higher than that in the mouth. For a quantitative research or diagnostic study of the thermoregulating mechanisms an electrical thermometer placed below the tongue in the closed mouth, in a well-covered external auditory meatus or in the œsophagus gives reliable measures of abrupt changes in arterial blood temperature (Cooper, Cranston & Snell, 1964a). These changes are poorly reflected in the rectum which, however, follows slow and prolonged temperature changes well. For ordinary clinical use a mouth temperature measured with a mercury and glass thermometer is satisfactory. It is important however to realize that instruments stamped with  $\frac{1}{2}$  min or 1 min rarely register the true temperature in that time. They take 5 minutes at least to give an accurate record and then only when the mouth is kept closed. Most of them have a scale commencing at about  $95^{\circ}\text{F}$  ( $35^{\circ}\text{C}$ ) which means that they cannot of course be of any use in a hypothermic patient. Using electrical measuring instruments which employ either thermocouples or thermistors, temperatures can be measured in many sites. For continuous registration over a 24-hour period the rectal temperature is usually the most convenient measurement. Œsophageal temperature is particularly useful in newborn infants who seem to tolerate an œsophageal tube extremely well, whereas in unconscious or immediate postoperative adults it may evoke vomiting and consequent inhalation of vomit. There is no site whose temperature can be equated accurately with that of the intracranial temperature receptors. The hypothalamus is perfused with a rich blood supply which carries off the considerable metabolic heat produced in the region (Randall *et al.*, 1963). Changes in hypothalamic blood flow as well as changes in arterial blood temperature can thus lead to thermal stimulation of the central regulating mechanism.

For details of electrical measurement of body temperature, the reader is referred to a review by Hill (1964).

### **What Control Mechanisms are Used?**

Though rapid adjustment of the amount of stored heat in the body "shell" can be considered an important aspect of thermoregulation, over a longer period of time the balance between heat gain and heat loss over the whole body is necessary for core temperature stabilization to occur. A short list of the routes of heat gain and loss are given below, and what is known of their control will be discussed briefly.

#### ***Heat Gain***

##### **(a) From the environment:**

1. By radiation; proportional to the difference in the fourth powers of the absolute temperatures of the skin and the radiating surface.

2. By conduction; proportional to the temperature difference between the air or water and the body surface.
3. By ingestion of warm food.

(b) The body's own heat production:

1. Basal or minimal obligatory metabolism.
2. Muscular activity—exercise or shivering.
3. Release of heat from “brown fat”.

### Heat Loss

- |  |   |     |
|--|---|-----|
| (a) Conduction.  | } | 70% |
| (b) Radiation.   |   |     |
| (c) Convection—important in the design of tropical or arctic clothing. |   |     |
| (d) Evaporation—540 g-cals per g water evaporated.                     | } | 28% |
| 1. Insensible perspiration from skin.                                  |   |     |
| 2. From respiratory tract.   |   |     |
| 3. From skin via sweat secretion.                                      |   |     |
| (e) Warming inspired air; heat loss in urine and faeces.               |   | 2%  |

The figures are approximate for a sedentary person in a temperate climate.

### Heat Gain

On the heat production side of the balance sheet the meaning of “basal metabolic rate”—B.M.R.—and its control have both come in for some criticism in the last decade. In clinical practice, there is some doubt as to whether the conditions under which B.M.R. is measured can be truly described as basal. A 12-hour fast is easy to achieve, few wards are equipped to make the measurement at the neutral environmental temperature—32–34°C (89.6–93.2°F)—and more seriously, the presence of a doctor with complex equipment is likely to evoke some degree of excitement. The ordinary clinical test would better be termed *standard metabolic rate*, and although it differentiates between frank hyperthyroidism or hypothyroidism and the normal, there are many patients in whom this measurement gives equivocal results. A very useful procedure has been the “*sedated*” *metabolic rate* (Fraser & Nordin, 1955), in which after a 12-hour fast the patient is given intravenous sodium amytal (up to 1 g) until the respiratory trace on the spirometer becomes regular in tidal volume and slope. The trace is readily readable and the slope in no doubt; the results are more consistent than the ordinary B.M.R. and standard figures for the norm in males and females at various ages were related to the results of Robertson & Reid (1952).

The basal body heat production depends principally upon the output of thyroid hormone, and controversy still rages as to whether this is significantly increased by long term exposure to cold. Another source of metabolic heat is the utilization of “brown fat”, which is found in the newborn, and recently suspected of being present in the adult human (Dawkins & Hull, 1964, 1965). Brown fat cells differ from those in other adipose tissue in at least two ways. First, each brown fat cell contains quite a large amount of cytoplasm in which there are numerous fat droplets whereas ordinary adipose tissue cells

have little cytoplasm containing a single fat globule. The ordinary fat cell contains few mitochondria but the brown fat cell contains many, the cytochrome pigments which they contain giving the tissue its characteristic colour. There is some evidence that the absence of brown fat may be of importance in the causation of hypothermia in the newborn and possible also in the aged. In the newborn, triglyceride molecules contained in brown fat cells undergo the complex cycle of hydrolysis into free fatty acid and glycerol, formation of a fatty acid coenzyme A ester and resynthesis to triglyceride, some of the ester being oxidized to regenerate ATP. The cycle is turned on by exposure to cold, and the result is release of energy in the form of heat.

Brown fat has rich blood and nerve supplies, and there is evidence that the energy producing cycle is set in motion, during cold exposure, by noradrenaline released at sympathetic nerve endings (Dawkins & Hull, 1964).

The other modes of heat production are shivering and voluntary exercise. The latter is an often forgotten but important response to the conscious appraisal of the environmental conditions. There is evidence that the initiation and control of shivering involves stimulation of receptors in the C.N.S. (Chatonnet, Tanche & Cabanac, 1960) and also in the skin. In the conscious rabbit, Downey, Mottram & Pickering (1964) showed that cooling the blood passing up one carotid artery could cause shivering even though the skin temperature remained above 34°C (93.2°F). Cooling the blood of patients with complete traumatic transections of their spinal cords in the cervical region, by immersing the legs in cold water, caused shivering in innervated muscles (Johnson & Spalding, 1964). This happened despite a mean skin temperature of 34–35°C (93.2–95°F) and absence of sensory pathways from the cooled limbs to the brain. On the other hand, sudden exposure of a nude man to a cold wind is well known to initiate shivering even though the central temperature at the time rises slightly. Thauer (1961) and his co-workers working on lightly anaesthetized dogs have adduced powerful evidence for the integration of peripheral and central cold receptor activity in the control of shivering. Thauer's view, which seems to be the most commonsense approach to date, was that in the intact animal shivering was normally initiated by cold receptors in the skin, and that its intensity depended on the actual level of the central temperature. A curious adaptive process was found in Australian aborigines (Scholander *et al.*, 1958). These people have the ability to sleep naked in the cold desert night, allowing their central temperatures to fall to 32–33°C (89.6–91.6°F) without visible shivering. This ability appears to be an inborn quality, but its mechanism is not known (Hammel *et al.*, 1959).

### Heat Loss Mechanisms

The control of heat loss from the skin is mediated by fine and coarse mechanisms. The fine control is achieved by adjusting the volume of blood flowing through the skin in unit time, and the coarse controls are the variations in the amount of clothing worn and sweating. Clothing adjustment is determined by conscious assessment of environmental temperature and humidity. The *fine* control of skin heat loss has been shown to depend upon both central and peripheral temperature receptors. Stimulation of either

type of receptor evokes a skin blood flow proportional to the applied stimulus.

### **Skin Blood Flow**

#### **Evidence for a Central Receptor mechanism**

The central temperature may be considerably altered by hard exercise and hot or cold baths. Following such activity it returns to its proper level (Lefèvre, 1911). This return to an apparent set point suggests a mechanism which "knows" what the central temperature should be, and it would be reasonable to expect that mechanism to be actuated by the temperature of the arterial blood. The evidence that such a mechanism exists comes from observing the peripheral vascular responses to changes in blood temperature in man. Pickering (1932) showed that vasodilatation in one arm followed immersion of the opposite hand and forearm in water at 44°C (111.2°F) with a delay of 5–10 minutes. If, however, the circulation to the heated arm was arrested no vasodilatation was evoked until the circulation was re-established. The extent of the vasodilatation appeared to depend on the magnitude of the rise in central temperature. Thus the vasomotor response was dependent on a rise in blood temperature and not upon a reflex from the heated skin. Later, Snell (1954) investigated these observations further in a quantitative way. He infused varying amounts of saline at 42–44°C (107.6–111.2°F) intravenously and measured the heat elimination (a measure of blood flow) from the opposite hand. He also measured the sublingual and rectal temperatures continuously with thermocouples. He was able to calculate the amount of heat infused into the subject. The area bounded by the rise and fall in oral temperature and the line which that temperature would have taken had no hot saline been infused was used as a measure of the total stimulation of the central "warm" receptors. A similar area measurement was used as a measure of the total response in hand blood flow. Snell (1954) found that the blood flow response to this type of body heating was linearly related both to the quantity of heat infused and to the area bounded by the rise and fall in central temperature. The order of magnitude of change in central temperature necessary to evoke an increase in hand blood flow was less than 0.2°C (0.36°F) and exceeded 0.05°C (0.09°F). A similar relationship has been found to apply to finger heat elimination following stimulation of central receptors both in response to infused warm saline and to heat supplied by immersion of one hand and forearm in warm water (Cooper, Cranston & Snell, 1965).

#### **Location and Nature of the Central Receptors**

A great deal of work on animals has indicated thermoreceptive structures in the hypothalamus (Keller & Hare, 1932; Ranson, 1940; Clarke, 1961; Freeman, 1961; Andersson, 1957; Folkow, Ström & Urnäs, 1949; Ström, 1950). In the cat, Kruger *et al.* (1959) also have shown an inverse correlation between the skin blood flow and the hypothalamic temperature. Some unpublished observations in man, in which warm saline has been injected into various arterial sites during diagnostic angiography, show that central warm receptors lie in the distribution of the internal carotid artery (Cooper, Cranston & Snell, unpublished data).

Some very elegant experiments on the cat by Von Euler (1964) showed that local heating of the preoptic and supraoptic regions of the hypothalamus evoked skin vasodilatation and a fall in body temperature—the ratio of the fall in body temperature to the rise in hypothalamic temperature being of the order of 8 : 1. Hardy, Hellon & Sutherland (1964) and Cunningham & Hardy (1965) have picked up electrical potentials from the fibres in the anterior hypothalamus some of which increase in frequency as the temperature is raised, and others which increase in frequency as the temperature falls. The nature and mode of stimulation of the receptor structures are not known with any certainty. Clinical evidence would also suggest that the hypothalamus is concerned with the central temperature regulating mechanism in man—*vide infra*.

The hypothalamus in animals and man contains noradrenaline and 5-hydroxytryptamine in high concentrations as compared with other parts of the brain (Vogt, 1954; Amin, Crawford & Gaddum, 1954; Bertler, 1961). These monoamines are contained in fine fibres and nerve terminals in many parts of the hypothalamus. Previous suggestions that these amines might be concerned in part in temperature regulation (Brodie & Shore, 1957; Von Euler, 1961) had substance added to them by the experiments of Feldberg & Myers (1963, 1964, 1965). They injected 5-HT into the lateral cerebral ventricle and the anterior hypothalamus of the cat, and this produced a prolonged rise in body temperature. Similar injections of noradrenaline lowered the cat's body temperature. The dog behaves similarly to intrahypothalamic administration of these amines. In the rabbit (Cooper, Cranston & Honour, 1965, Ruckebusch, Grivel & Laplace, 1965, 1966) and the sheep (Bligh, 1966b) the response to intraventricular noradrenaline is a rise in body temperature, and 5-HT causes a fall—the converse of the effects in the cat and dog. 5-HT given into the lateral cerebral ventricle causes a fall in temperature in the ox (Findlay & Robertshaw, 1967) and the goat (Andersson, Jobin & Olsson, 1966). Cerebrospinal fluid has been pumped from the third ventricle of one monkey into the third ventricle of another (Myers, 1966). When the donor monkey was heated strongly the temperature of the recipient monkey fell and vice versa. The substances responsible for these effects have not yet been identified. There are many plausible explanations of all these findings, one of which suggests that the two amines are the transmitter substances released at central thermoregulatory synapses. There is as yet no experimental proof of this hypothesis, which might also apply to a number of other functions since the amines occur in hypothalamic fibres probably subserving a number of different central control mechanisms.

#### **Evidence for the Peripheral Receptors**

An immediate vasoconstriction occurs in one upper limb as the result of immersion of the other in cold water (François-Franck, 1876). This response still occurs if the circulation to the cooled limb is occluded, and is therefore a nervous reflex mechanism. The reflex fatigues within a few minutes.

Radiant heat directed on the trunk and abdomen evokes a vasodilatation in the hands which commences within 10–15 sec of application of the heat. A similar vasodilatation occurs in the hand when the front of the legs is

heated, and it is not abolished by occluding the leg circulation (Kerslake & Cooper, 1950). Thus the vasodilatation is evoked by an alteration in the pattern of nervous impulses arising in the heated skin. It is frequently accompanied by a fall in central temperature. The conditions under which this reflex may be evoked are that the central temperature must be above  $36.5^{\circ}\text{C}$  ( $97.7^{\circ}\text{F}$ ) and the mean skin temperature above  $32^{\circ}\text{C}$  ( $89.6^{\circ}\text{F}$ ) (Cooper, Johnson & Spalding, 1964c). Below these temperatures, the reflex becomes progressively inhibited in healthy subjects. It has also been demonstrated that the magnitude of the reflex vasodilatation response is quantitatively related to the applied heat load (Cooper, 1965a). The afferent fibres concerned in this reflex probably travel with the sympathetic nerves, and they are not identical with the fibres conveying the consciously appreciated thermal sensation. The pathway within the central nervous system is not known, but the reflex arc extends above the medulla oblongata (Appenzeller, 1965, personal communication).

### **Sweating**

On the control of sweating there is still considerable controversy. There seems ample evidence that both central and peripheral receptors are concerned in the determination of the rate of sweat production. Benzinger (1959) attributed most if not all of the control of sweating to the central mechanism. Kerslake (1955, 1961) in very elegant experiments showed that the sweat rate was linearly related to the calculated temperature of hypothetical receptors assumed to be situated at the level of the sweat gland. Also he demonstrated that sweat loss from the arm followed cyclical variations in heat applied to the trunk with a short time lag. This was so brief that blood from the heated region could not have reached any central nervous structure in order to initiate the change. A reflex component must therefore be assumed.

### **Local Effects of Temperature**

Blood flow in a sympathectomized hand or foot can be varied by immersing it in water of differing temperatures. The response is similar to that seen in the normal limb. Blood flow rises gradually as the local temperature is raised between  $12$ – $32^{\circ}\text{C}$  ( $53.6$ – $89.6^{\circ}\text{F}$ ). Above  $32^{\circ}\text{C}$  ( $89.6^{\circ}\text{F}$ ), the rise of blood flow for each increment in temperature becomes much greater (Greenfield, 1963). However, immersion of a hand in water at  $0$ – $6^{\circ}\text{C}$  ( $32$ – $32.8^{\circ}\text{F}$ ) evokes a series of waves of cold vasodilatation, originally noticed by Lewis (1930). This response is not dependent on an intact nerve supply to the hand (Greenfield, Shepherd & Whelan, 1952). A very large proportion of the body's heat production can be lost from the hand under these circumstances. The response is, in healthy subjects, to some extent related to the thermal state of the rest of the body, being smaller in a cold than in a warm person. The mechanism is in part due to the direct effect of low temperature in paralysing the arterial smooth muscle (Keatinge, 1961), and its extent depends on the basal state of the finger vasomotor tone. This phenomenon is of considerable importance in determining survival during whole body immersion in very cold water.

### Countercurrent Heat Exchange

Bazett *et al.* (1948) originally suggested, and Scholander (1958) extended, the concept that heat exchange could take place between closely adjacent arteries and veins, or arterial and venous plexuses. For example, in the cold, the venæ comites associated with the radial and ulnar arteries carry a substantial quantity of blood. Heat is lost from the arterial blood to the cooler venous stream. There is no nett loss of body heat; and the result of the precooling of the arterial blood will be to reduce the temperature of the arterial blood arriving in the skin capillaries, thus diminishing the loss of heat to the environment. In the heat, the venæ comites carry little blood, which is diverted through superficial veins with a converse effect on heat loss.

## DISORDERS OF TEMPERATURE REGULATION

The body temperature may be abnormally high (fever)\* or abnormally low (hypothermia). "Physiological" fever occurs during strenuous exercise, immersion in a hot bath, or when working in the heat wearing impervious protective clothing. Subnormal body temperature is a common finding early in convalescence from a severe febrile illness (Lèfevre, 1911). While the range of normality for body temperature is well defined for young healthy adults, little information is available on the range of body temperature in early childhood or in the aged.

### Fever

#### Pyrogens

The mechanism of fever has been most closely studied in fever caused by Gram-negative organisms, particularly the salmonella group. This is because the fever producing agent can be extracted from these organisms with relatively simple chemical techniques. This substance, called bacterial pyrogen by some and endotoxin by others, is of large molecular size and is a lipopolysaccharide. It can be extracted and highly purified (Westphal & Lüderitz, 1954; Work, 1965, personal communication). Whether the pyrogenic properties derive from the lipid or from the polysaccharide part of the molecule is still a matter of controversy. These lipopolysaccharides are extremely potent, a high fever being caused by the intravenous injection of as little as  $1 \times 10^{-2}$  to  $2 \times 10^{-2}$  ng according to the origin of the material. Work, 1965 (personal communication) has shown that the *E. coli* lipopolysaccharide is excreted in "packets" from the interior of the organism when it is grown in a lysine free culture medium.

When the bacterial pyrogen is injected into the circulation, it interacts with leucocytes, and these liberate another substance into the plasma called leucocyte pyrogen or endogenous pyrogen (Grant & Whalen, 1953; Bennett & Beeson, 1953; Cranston *et al.*, 1956). Leucocyte pyrogen differs in some biological properties from bacterial pyrogen. It is heat labile being destroyed at 60°C (140°F) whereas bacterial pyrogen requires dry heat at 170°C (338°F) for several hours for its destruction. Repeated daily intravenous injection of bacterial pyrogen leads to a state of "tolerance" in which the febrile response to a given dose becomes greatly reduced. In this tolerant

\* 'Hyperthermia' is acceptable, but 'fever' is preferable by custom and long usage.

state, leucocyte pyrogen evokes a fever of the same order of magnitude to that seen in the normal animal. In man, leucocyte pyrogen causes fever which starts in 15–20 minutes after intravenous injection whereas the latency following bacterial pyrogen is some 60–75 minutes. The chemical nature of leucocyte pyrogen is not known, though recent chemical analysis has shown that it has a molecular weight between 10,000 and 20,000 (Rafter *et al.*, 1960). It is now almost generally agreed that in infectious fevers, leucocyte pyrogen is the substance which acts within the central nervous system to disturb the thermoregulatory system (Atkins, 1960). Atkins & Snell (1963) have demonstrated that a pyrogen, having similar properties to leucocyte pyrogen, can be extracted from muscle and other tissues under completely aseptic conditions. This provides some basis for our understanding of non-infective fever, for example that following cardiac infarction.

### **The Site and Mode of Action of Leucocyte Pyrogen**

The events accompanying the onset of fever are intense vasoconstriction in the skin, piloerection, shivering, headache, and of course a coincident rise in body temperature. The peripheral vasoconstriction has proved a useful index of the disturbance of temperature regulation in experimental fever. The source of this increased vasomotor tone has been investigated both in man and animals over the past few years (Cooper, 1965a). It does not derive from a derangement of the reflex mechanisms which depend on cutaneous receptors in man (Bryce-Smith *et al.*, 1959). Leucocyte pyrogen does not act on the spinal cord or anywhere in the peripheral part of the sympathetic nervous system (Cooper, Johnson & Spalding, 1964b). It does act, in man, above the cervical level of the cord, and probably intracranially. The evidence as to its mechanism of action in man supports the view that the central receptors operate in fever at their normal sensitivity, but at an unusually high threshold. In other words, the set point of the mechanism is raised (Cooper, Cranston & Snell, 1965). Evidence from observations on patients with absent thermoregulation, but with intact baroreceptor and emotional vasoconstrictor pathways, indicates that these latter neurones are not affected by leucocyte pyrogen (Hockaday *et al.*, 1962).

The search for the site of action of leucocyte pyrogen has been carried out in rabbits (Cooper, 1965b; Cooper, Cranston & Honour, 1967). There is only one part of the brain which responds to infusion of leucocyte pyrogen by a marked rise in body temperature. This region is sharply located to the anterior hypothalamic and preoptic areas, close to the wall of the third ventricle. The same site of action has been found in the cat by Jackson (1967). In this part of the brain there are abundant monoamine-containing neurones. It is tempting to postulate that leucocyte pyrogen could release the appropriate amine from these neurones and that the amine could be the final mediator of the febrile response. The evidence is as yet inadequate to substantiate or refute this hypothesis.

It is of interest to note that monoamine oxidase inhibitors can alter the pattern of pyrogen induced fever and in combination with other drugs induce fatal hyperpyrexia in the rabbit (Cooper & Cranston, 1966; Loveless & Maxwell, 1965).



### **Clearance of Bacterial Pyrogen from the Body**

There is ample evidence that bacterial pyrogen is cleared rapidly from the circulation. Some is taken up by leucocytes and platelets which then alter their surface properties and adhere to capillary walls particularly in the lung and liver. Much is transferred to the reticuloendothelial cells (Cooper & Cranston, 1963). Labelled bacterial pyrogen is excreted in bile and urine and probably by other routes. The development of *tolerance* is accompanied by more rapid clearance of bacterial pyrogen from the circulating blood (Cooper & Cranston, 1963) but the mechanism of tolerance is unlikely to depend solely on this increased rate.

### **Other Effects of Circulating Pyrogen**

*Renal and Splanchnic Circulation.* Chasis *et al.* (1938) found that a rise in effective renal plasma flow followed an intravenous infusion of pyrogen contaminated inulin. This effect of bacterial pyrogen has been confirmed (Cranston, 1959). The kidney blood flow response is independent of the nerves to the kidney and not due to a direct effect of bacterial pyrogen on the kidney since it occurs in a transplanted kidney after intrathecal administration of pyrogen (Cooper *et al.*, 1960). It must depend on a substance released either from an intracranial source or from an organ whose nerve supply is stimulated by the central action of pyrogen. There is a similar increase in total splanchnic blood flow (Bradley *et al.*, 1945). When one considers the large proportion of the cardiac output passing through the splanchnic circulation, an increase of 50–70 per cent must be of great clinical significance. This is particularly true of infections in patients with low output cardiac failure.

*Headache.* It was of interest to note that a patient with a complete traumatic section of the spinal cord at C-6 did not get headache following intravenous pyrogen although he shivered violently in his few normally innervated muscles (Cooper, Johnson & Spalding, 1964b). There are many observations that headache in fever can be relieved by evoking a generalized vasodilatation. The evidence suggests that the headache of fever is in some way related to the intense vasoconstriction and not due to the direct action of pyrogen on pain producing structures within the cranial cavity.

### **Unusual and Factitious Fevers**

A number of patients either falsify the thermometer readings by keeping a spare clinical thermometer which they substitute for the one given them by the nurse or they take drugs such as thyroid hormone and dinitrophenol to cause them to have high body temperatures. Some of them do this in order to have repeated medical examinations so that they can reassure themselves but in others the psychological reasons for their behaviour are complex and obscure.

Hysterical fever can occur, and has been noticed, on occasion, to be accompanied by hyperventilation against a partially closed glottis. This has been shown to raise the body oxygen uptake considerably. Knowing that the hypothalamic structures can affect breathing, and that they are closely associated with emotional responses, it is tempting to suspect an organic basis for this disorder.

We have seen a patient whose fever remained unexplained after several years of investigation. Her oral and oesophageal temperatures were abnormally high and her rectal temperature was within normal limits—usually lower than the oral temperature (Cooper, 1965a). Experiments suggested that she might have an abnormal distribution of body heat mediated via the sympathetic outflow, and there was E.E.G. evidence of deep seated abnormality in the brain. She had no tumour or vascular abnormality in her brain and the tentative suggestion that the thermal problem was a sequel of a mild encephalitic attack remains possible but unproven.

Luft and his colleagues, in Stockholm, have described a most interesting cause for a raised basal metabolic rate in which the thyroid hormone secretion is not involved (Ernster, Ikkos & Luft, 1959; Ernster & Luft, 1962; Ernster & Luft, 1963; Luft *et al.*, 1962). Studies of the mitochondria, obtained from human muscle biopsy specimens, showed that they were “hyperactive” leading to a great energy release, and not under the usual thyroid hormone control. The exact cause of the hypermetabolic activity of these mitochondria is not clear, but the authors postulate a possible enhancement of protein and respiratory enzyme synthesis. The natural history of this disorder is not known.

High body temperatures occur from time to time during prolonged heat exposure. For a summary of “heat illnesses” the reader is referred to Leithead & Lind (1964). A predisposing cause of anhydrotic heat exhaustion was found by Bannister (1959, 1960) who showed that circulating bacterial pyrogen would inhibit sweat secretion. A few patients suffer congenital anhydrotic ectodermal dysplasia (Cockayne, 1933). Here there is complete or partial loss of sweat glands. This was thought to be due to an X-linked recessive gene in families where males alone are affected. There is recent evidence that in some cases where females also are involved, there could be tissue mosaicism dependant on an inactive X-chromosome (Kerr *et al.*, 1966).

Finally, there are the curious familial periodic fevers (Reimann, 1951) which are still unsolved. Fever occurs at fairly regular intervals, and is often associated with rashes or inflammatory affection of joints. No completely satisfactory answer has been found to this problem, though the presence of abnormal steroids in the plasma (e.g. unconjugated ætiocholanolone) may be a part of the story (Kappas *et al.*, 1960). Bondy, Cohn & Gregory (1965) have reviewed the pyrogenic steroid problem. Degradation products of testosterone and androstenedione are androsterone and ætiocholanolone. These compounds are said to be pyrogenic, but lose the fever producing property if the 3-hydroxyl group is either esterified or conjugated. Recent evidence of Bodel and Dillard (1966) suggests that, in man, the unconjugated steroid can stimulate leucocytes to produce leucocyte pyrogen. It is possible that at sites outside the liver ætiocholanolone can be produced *de novo*, or acted on by enzymes which split the conjugated form.

### Hypothermia

A lowered body temperature may result either from a low body heat production or an abnormally high heat loss in relation to typical environmental conditions; or to exposure to such extremes of environmental

conditions that the heat conservation mechanism is inadequate to sustain enough body heat. There are chronic and episodic forms of this disorder.

### **Excess Environmental Stress**

This follows immersion in cold water, or prolonged exposure in cold or cold wet conditions usually accompanied by severe fatigue. Immersion hypothermia was first studied scientifically by James Currie (1798). He was the first to observe a continuing fall in body temperature in a subject after removal from cold water, and he also noted the slowing of the pulse rate which accompanied a drop in body temperature. Much information has been gleaned during and since the last war by the navies involved concerning immersion hypothermia. It seems clear now that survival of the average man for more than seven hours in water at 15°C (59°F) is rare, and 30 minutes is the maximum survival time in water at 0°C (32°F). Survival in very cold water can be prolonged by two factors which at first sight seem subversive to good sense. Clothing, even if it is not waterproof, provides sufficient thermal insulation to provide a significant retardation in the rate of fall of body temperature. Swimming *increases* the rate of heat loss in very cold water, and accelerates the death of the immersed victim (Keatinge, 1964). In very cold air survival times are much longer than in cold water. It is probable that fatigue not only prevents the victim from constructing a suitable shelter but also interferes in some way with the thermoregulatory defence mechanisms against cold. It is therefore sound advice that people caught out in the open in extremely cold weather would do better to construct some form of shelter and to stay in it before they have reached the condition of extreme fatigue.

### **Reduced Body Heat Production and Increased Heat Loss**

This may occur as a result of reduced thyroid hormone output, or in the metabolic failure of a diabetic coma. Any cerebrovascular accident which destroys the shivering mechanism or other heat conserving functions can lead to hypothermia. Paralysis due to trauma especially at a high spinal level, drugs and poisons such as chlorpromazine, barbiturates, alcohol and carbon monoxide, are all recognized as capable of leading to a severe hypothermic state. The problems of neonatal hypothermia (Mann & Elliott, 1957) and cooling during anaesthesia have been dealt with recently (Hunter, 1964). In old people the problem is partly sociological and partly medical. There are in Britain a large number of old folk who live in very squalid conditions. While the sociological conditions undoubtedly contribute to the incidence of hypothermia in the aged, there are many examples of old people suffering hypothermia in the excellent care of considerate relatives. Relatively few houses in Britain have central heating and it is our custom to keep our bedrooms at extraordinarily low temperatures: under these conditions the elderly can be seriously at risk. The number of aged people at risk continues to rise. Studies on old people who have survived hypothermia show a high incidence of postural hypotension, and the occurrence of selective loss of parts of the heat conservation mechanisms—e.g. loss of shivering with intact vasoconstrictor responses (Johnson, Macmillan & Wollner, 1966, personal communication). The nature of the degenerative changes concerned

have not yet been confirmed. The dietary balance and requirements of old folk may not be met by the standard figures worked out for healthy young adults because for example of possible changes in intestinal absorption, and a survey of the dietary intake and some careful absorption studies would be worth while in people over 65 years of age.

### **Important Physiological Changes in Severe Hypothermia**

The altered physiology of hypothermia has been reviewed frequently in recent years (Cooper, 1959; Brewin, 1964; Nisbet, 1964), and it is proposed here to mention only some of the salient features of direct clinical interest.

*Metabolism.* In the absence of thermoregulatory responses, the metabolic rate falls by a factor of approximately two for a 10°C (18°F) drop in body temperature (Cooper, 1965a). However, when thermoregulatory mechanisms are intact, exposure to cold evokes shivering which, as the body temperature falls below about 31–32°C (87.8–89.6°F), gives way to intense muscular rigidity. Pulmonary ventilation may be severely reduced leading to a high arterial pCO<sub>2</sub> and a low arterial pO<sub>2</sub>. This latter condition is of interest in that the respiratory drive may derive from the anoxæmic stimulus, and administration of oxygen without artificial ventilation can lead to respiratory arrest; and artificial ventilation may be required.

Another feature of interest is a severe impairment of glucose utilization at body temperatures of about 30°C (86°F) and below (Wynn, 1954). This occurs even in the presence of large amounts of circulating insulin and appears to result from an inhibition of the glucose carrier systems of the cell membrane.

*Acid-base Balance.* There is usually some degree of acidosis in severe hypothermia, and this may become of more importance during the rewarming process. The acidosis may be respiratory due to CO<sub>2</sub> retention as a consequence of reduced pulmonary ventilation. There is usually some additional metabolic acidosis derived partly from lactic acid production in shivering or contracted muscle, partly from tissue respiration in regions having an inadequate blood flow and also from a decreased capacity of the liver to deal with acid products of muscle metabolism.

*Circulation.* There is usually a progressive fall in heart rate as the body temperature falls, but this occurs without the expected prolongation of the relative diastolic “rest” period. Cardiac output and arterial blood pressure fall, the latter drop being smaller than expected from the cardiac output change because of increased peripheral resistance. Increased peripheral resistance occurs partly due to vasoconstriction, but a larger component is an increase in blood viscosity due to low temperature and hæmoconcentration. In the electrocardiograph, the P-R, Q-T, and QRS intervals are lengthened. The S-T segment is frequently elevated and the T-wave may eventually be inverted. The so-called “J-wave”, an odd spike or elevation in the R-S wave is frequently seen and seems to have little prognostic significance. At any temperature below 30°C (86°F) ventricular fibrillation may occur. The variation in temperature at which this can occur and death ensues is great, and the concept of a “lethal temperature” is unsound.

*Other Changes.* There is impairment of bromsulphthalein (BSP) excretion and it is possible that the ability of the liver to detoxify and excrete drugs may be greatly reduced. A rise in central venous pressure is known to bring

about cellular damage in the liver during hypothermia (Brewin, 1964). Renal function is generally reduced. Urine flow may be less diminished in spite of reduced glomerular filtration by reason of the suppression of tubular reabsorption.

In the central nervous system, hypothermia generally suppresses function, but the temperature at which consciousness is lost or even retrograde amnesia occurs is widely variable (Cooper & Ross, 1960; Cooper, 1964). Afferent nerves have a higher threshold for stimulation than usual, but the action potentials evoked are of greater magnitude and duration than at normal temperatures.

### **Treatment of Hypothermia**

The rules governing the care of unconscious patients are frequently forgotten because the use of the jargon word "hypothermia" distracts from attention to the mundane details of medical care. The basic nursing care of these people does not differ from that of any other unconscious patient. Diagnosis of the underlying cause may present great difficulty at low body temperature. A very rapid heart rate in hypothermia sometimes indicates internal hæmorrhage. A raised blood sugar or glycosuria may result from severe hæmoconcentration or impaired renal function and not indicate a diabetic state. Myxædema, a diagnosis of which is often difficult to prove in a severely hypothermic patient, may become apparent during slow rewarming by a failure of metabolic heat production and hence no rise in body temperature. Measurements of protein bound iodine or  $T_3$  resin uptake tests frequently cannot be done in time to be of much value in the early treatment of an acute hypothermia. They may also not be satisfactory if blood is taken from a sequestered pool in an area of low blood flow where the state of hæmoconcentration or hæmodilution are not representative of that in properly circulating areas. These measurements are of course of use in subsequent diagnosis and treatment of the patient. In all cases the chances of survival decrease with the duration of the hypothermic state. Two methods of rewarming are currently used. In the first, or slow method, the patient is covered with blankets and preferably placed in a room at 26.4–29.4°C (80–85°F). Further heat loss is prevented and the patient's temperature rises by means of his own metabolic heat production by 1–2°C (1.8–3.6°F) per hour. This method is recommended in old people and if the duration of hypothermia has exceeded 12 hours. The other technique, of particular use in young people suffering immersion hypothermia, is immersion in a hot bath at 42–43°C (107.6–109.4°F). In this method, the immersed patient will obviously be kept head up, and care has to be taken that the blood pressure does not fall to levels which would cause cerebral anoxia. The use of nor-adrenaline and other vasoconstrictor agents is dangerous. Two other methods are being tried. First, immersion of a forearm in water at 43°C (109.4°F) while the rest of the body is heavily insulated has been found to raise the body temperature some 3°C (5.4°F) per hour. The use of a natural heat exchanger to provide blood stream warming would theoretically lead to a warming of the heart and hence a useful increase in heart output. To obtain greater vasodilatation and heat exchange in the immersed arm only, an injection of 5 mg papaverine into the brachial artery is of use. Others (Hart,

1965, personal communication) are trying the effect of the mini-coil artificial kidney as a combined heat exchanger and electrolyte corrector and the results look promising.

There are some special problems in rewarming.

*Respiratory.* In view of the respiratory acidosis previously mentioned, artificial ventilation of the lungs with or without tracheotomy may be required to reduce the  $p\text{CO}_2$ . Pulmonary oedema is commonly seen during rewarming in the older age groups.

*Electrolytes.* In some patients there are changes in plasma electrolytes and in others there are not. The electrolyte levels in *arterial* blood only are important. Blood in veins may, during hypothermia, represent stagnated sequestered pools. We have found some instances in which arterial and large vein samples differ widely in their electrolyte contents. The danger is that the correction of an imbalance shown in a stagnant venous sample may be the correction of an artefact which is irrelevant to the arterial circulation and lethal to the patient. Despite previous forebodings (Cooper, 1964) it seems that careful arterial puncture, using a fine needle and plenty of local anaesthetic round the vessel, is quite safe.

If shivering occurs or there is intense muscle tension present then metabolic acidosis will probably be present. This can be corrected with intravenous bicarbonate or one of the more modern buffers (e.g. tris). It is not uncommon in old people suffering from severe hypothermia to see the rapid development of oedema over a great deal of the body surface. This can occur with great rapidity and even in the presence of an extremely low arterial blood pressure. One therefore suspects that many capillaries can become leaky and in this condition the administration of intravenous fluids could be very dangerous. Because it is known that the secretion of adrenal cortical hormones is considerably reduced in severe hypothermia many advocate intravenous administration of hydrocortisone. There is as yet insufficient evidence that this is of any value.

*The Hypothermic Diabetic.* A special point of interest is the diabetic in coma whose body temperature falls below  $30^\circ\text{C}$  ( $86^\circ\text{F}$ ). Here, Wynn (1954) has shown that glucose is not utilized in the non-diabetic, and that administered insulin has little or no effect. It is best to raise the body temperature rapidly to  $31\text{--}32^\circ\text{C}$  ( $87.8\text{--}89.6^\circ\text{F}$ ) and then administer insulin.

*Hypothyroid and Hypopituitary States.* These have been mentioned frequently elsewhere (Cooper, 1961). The danger that excess triiodothyronine may precipitate signs of coronary insufficiency is emphasized.

### Chronic and Episodic Hypothermia

More and more cases are coming to light of patients whose body temperatures are chronically subnormal, sometimes grossly so. There appear to be two types (Hockaday *et al.*, 1962). Some patients have lost all control of body temperature except the conscious appreciation of whether their surroundings are hot or cold—and so can vary their clothing. Others definitely regulate their temperatures at very low levels. This latter type can occur in an episodic form (Duff *et al.*, 1961). As yet, the correlation of these findings with C.N.S. pathology is not available. It is suspected that such events could result from damage to the thermoregulatory mechanisms at

many points. The patient may present with a story suggestive of an intracranial lesion which then resolves and leaves the patient with a low body temperature. There may be a gradual onset of mental deterioration or retardation, or this may occur in episodes where the body temperature is low. Excessive sweating may occur in the hypothermic phase of the episodic form. Examination of the patient, particularly the elderly person, after repeated admissions with low body temperature may show selective loss of one or more of the heat conserving mechanisms; and this may or may not be associated with evidence of more widespread loss of function of the autonomic nervous system. Only careful, quantitative measurement of all aspects of thermoregulation can establish the diagnosis. Finally, considerable thermoregulatory disturbance may be encountered in patients with widespread erythrodermic skin conditions (Fox *et al.*, 1965). There may be fever, but the fixed dilated skin blood vessels can lead to a precipitated fall in body temperature in a cold environment. The basal metabolic rate is said to be raised without thyroid hormone oversecretion, but the discomfort of the condition makes a B.M.R. evaluation difficult, and may explain the slight rise in vanillyl mandelic acid excretion in the urine. )

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## CHAPTER 12

# ENVIRONMENTAL FACTORS IN DISEASE

by

E. G. KNOX

### The Concept of Cause

THAT all diseases have causes is a proposition at once obvious and difficult to justify, but if we define our terms carefully it would appear intuitively acceptable to most people. However, many doctors and medical students develop an attitude, hardened by a daily need for simple explanations, which admits only that *some* diseases (diabetes? thyrotoxicosis? tuberculosis?) have causes while others (road accidents? malformations? appendicitis?) just happen. They might not defend this as an explicit point of view if they were challenged but might suggest that for some diseases we know the "actual cause(s)" while for others we can only identify "contributory factors". But what exactly is a factor, and how does it differ from a cause?

Clearly, this difficulty is related to the now traditional distinction between pathogenesis and ætiology. The first is the connected sequence of internal events and processes which produce the clinical features of the disease. The second is the external pattern of events and circumstances which determine that one individual gets the disease while another does not. The first is of interest chiefly to doctors who specialize in the interruption and modification of pathological processes by manipulating the internal environment. The second is of interest chiefly to those whose job is to alter the risk of disease, through manipulating the external environment. Therefore the distinction between pathogenesis and ætiology is a convenient one in that it is suited to the current organization of medical practice. However, it is not difficult to find examples which confuse the issue. A pathogenic organism is first an external and later an internal cause of disease while deficiency diseases provide similar problems.

### Necessity and Sufficiency

A more fundamental way of thinking is in terms of the classical division of causes into those which are necessary and those which are sufficient (and those which are both or neither). A necessary cause is a cause which *must* have been present if its consequence followed; a cause is not a necessary cause when an alternative cause has the same effect. A sufficient cause will lead to its consequence whatever the other circumstances might be; a cause is not a sufficient cause when a combination with another is a condition of its effectiveness.

Necessary causes (mosquitoes, *Myco. tuberculosis*) and sufficient causes (a bullet, the gene for achondroplasia) are traditionally incorporated within the pathogenesis of a disease while those which are neither necessary nor

sufficient (cigarettes, fog, prenatal X-rays) are incorporated in the ætiology and are frequently referred to as contributing factors. However, the nature and origins of non-sufficiency and non-necessity must be emphasized; these characteristics arise for combinatorial reasons.

The use of the term contributory factor must not mislead us into thinking that such causes are fundamentally different from other causes. They are non-necessary simply because an alternative pathway will serve and non-sufficient because other causes must be present too. The combinatorial aspect justifies nicely the use of the term "factor", with its multiplicative connotation, for the probability of a combination of independent events occurring in sequence is the product of the probabilities of occurrence of the events singly.

The concepts of necessary and sufficient causes are discussed in two recent works on epidemiology by Morris (1964) and by MacMahon, Pugh & Ipsen (1960). Morris (pp. 190-91) uses the term "necessary cause" in a less rigorous sense than that implied above. He speaks of "*a* necessary cause, *the* specific agent", whereas there may be several; alternatively there may not be any. MacMahon, Pugh & Ipsen (1960) seem to take the view that the whole cause-effect concept is not a very deep one anyway. In discussion of necessary causes for example they raise the interesting point that a necessary cause may be necessary simply by definition.

As an example let us consider two diseases, rhesus hæmolytic disease of the newborn and ABO hæmolytic disease of the newborn. In each case an appropriate maternal-fœtal blood group combination is a necessary, although not a sufficient, cause. Suppose however we were to detect a cause common to both diseases, for example the passage of fœtal red cells into the maternal circulation, we could define the combined disease as fœtal-maternal transfusion sickness. With the new definition neither of the particular blood group combinations is now a necessary cause.

MacMahon, Pugh & Ipsen (1960) also quote Hume who wrote in 1739 "We are never able, in a single instance, to discover any power or necessary connection, any quality which binds the effect to the cause, and renders the one an infallible consequence of the other. We only find that one does actually, in fact, follow the other." They imply that the cause-effect inference is essentially pragmatic and related to our ability to manipulate a situation.

### **Inference from Association**

Whatever importance we care to put upon causes we can at least agree that they are neither directly demonstrated, nor logically deduced, but inferred. Inference (or induction) is the process of arguing from the particular to the general, in this case the interpretation of observed associations between events. Inference is never a logically water-tight procedure, in contrast with a well-constructed deductive argument where we proceed from the general to the particular, as in the deduction of geometric theorems from axioms. In epidemiological work the problems are especially severe because the interest is chiefly with non-necessary and non-sufficient causes and they must be inferred less directly and less certainly than the necessary or the sufficient. Indeed, for some statistical associations a causal interpretation may not seem possible.

For example, whooping cough is notified more often in girls than in boys. Femininity is certainly neither a necessary nor a sufficient cause of whooping cough nor is it easy to say how it contributes. We may speculate that the pattern of transmission is such that it interacts with the different social- or play-patterns in boys and girls, or that for a given degree of exposure boys have a more effective mechanism of resistance, or that for a given level of symptoms the parents of a girl will call the doctor more often than will the parents of a boy. It may be that male doctors, educated for the most part in exclusively male institutions respond in the presence of a distressed little girl with a certain insecurity and a compensating diagnostic precision not felt necessary in the presence of a small boy. In this and in similar cases we are not in a position to make a cause-effect inference and if we use the term "factor" we do so in the vaguest of senses. The ambiguous overtone of causal speculation is most undesirable and we would do well in such situations simply to call an association an association.

At least we can say that the whooping cough was not a cause of the femininity. This is a more instructive statement than it appears at first sight because it illustrates the thesis that causal interpretations of demonstrated associations are always based upon external evidence. This is true of all interpretations of statistical associations and contingencies. In a purely formal sense an association between the frequencies of two events, A and B, is quite symmetrical and it may be that A causes B, that B causes A, or that both result from common causes. There is no intrinsic information to distinguish between these possibilities nor to guarantee that they are an exhaustive list of possibilities. Therefore the interpretation of the association must *always* be on the basis of evidence external to it. This is fundamental. By contrast, statistical significance tests and measures of the strength of an association are based entirely upon the observations without any reference whatsoever to external evidence. The use of mathematical statistical procedures in the interpretation of associations, when there is no apparent logical connection or communication between the processes of demonstration and interpretation is fraught with danger and ambiguity; there may be an intractable logical absurdity.

### CONTINGENCY METHODS

We have seen that, philosophically and operationally, the inference of causes from demonstrated statistical associations has been a major pre-occupation of epidemiologists in recent years. The usual presentation of such an association is in the form of a contingency table in which persons or observations are characterized simultaneously according to two (or more) systems of exclusive attributes. Individuals are located in the cells of a table according to the particular combination which they exhibit. The simplest form of contingency table is the so-called  $2 \times 2$  table expressing the four possible combinations of two simultaneous dichotomies. The pattern most familiar to doctors, satisfying also their more dramatic moments of self imagery, is the format of the idealized therapeutic trial, . . . Treated/Untreated v. Alive/Dead. An example close to this idealized form, adapted from the work of Brown, Stone & Sutherland (1966) is shown in Table 12.I.

TABLE 12.I

EFFECT OF BCG VACCINATION UPON THE DEVELOPMENT OF  
LEPROSY IN TUBERCULIN-NEGATIVE CHILDREN UP TO FIVE YEARS OLD

	<i>Unvaccinated</i>	<i>BCG vaccinated</i>	<i>Totals</i>
Leprosy	27	4	31
No leprosy	4550	4474	9024
Totals	4577	4478	9055

Not only has the contingency method dominated recent epidemiological thinking; it has come to be associated almost uniquely with the inference of causes. Yet enough has been said in introduction to cast doubt upon its special prowess in this respect, and its claims to usefulness seem to be on all fours with those of non-contingency analyses and of non-statistical methods. It will be the main thesis of what follows that the successes of the contingency method have always depended upon the economy and specificity of the hypothesis invoked to explain an observation, rather than upon the doubtful logic of the statistical manipulation.

This will be illustrated first by examining a group of contingencies which have been interpreted in a relatively exact manner, and later by examining some less exact interpretations. We shall begin with a group of iatrogenic diseases, then examine the evidence on coronary thrombosis, and finally describe some developmental studies in children.

### **Iatrogenic Disease**

The more important of the medically induced diseases detected in recent years on the basis of population studies as opposed to observations on individuals, are the effects of thalidomide leading to congenital malformation (phocomelia), pre-natal X-rays leading to leukæmia in childhood, irradiation of the thorax in infancy leading to carcinoma of thyroid at puberty, and the radio-diagnostic use of thorium leading to malignant disease of the liver. In each case the first has been shown to be statistically associated with the second; where A has occurred B has followed more often than when A did not occur, or, alternatively, when B was found, A has preceded it more often than when B was not found. The interpretation of these associations is based upon external evidence and it is clear that we can proceed methodologically in one or two directions. We can detect the association and try to explain it, or we can expect it on other grounds and then try to demonstrate it formally. From the point of view of seeking other iatrogenic illnesses it is of interest that except for the thyroid carcinoma, all the above associations appear to have been demonstrated in the second way. This applies also to other notable examples of iatrogenic disease, such as mercury and pink disease, or oxygen and retrolental fibroplasia.

The incidence of phocomelia in a group of obstetric departments in West Germany was reported as 0.6 per 1,000 births in the period 1950–57. This leaped to 1.3 per 1,000 in 1960, 2.7 in 1961 and 2.9 in the first part of 1962

(Lenz, 1963). It was primarily from this observation of an increased frequency, together with the prior knowledge that malformations may be induced experimentally in animals by administering a wide variety of chemical substances, that investigation of the identity of the agent proceeded. Association of the drug with the defect was used to specify and present the situation more precisely, and withdrawal of the drug eventually confirmed the causal association.

The production of malignant disease through irradiation, in animals, radiologists, and luminizers, was known long before the Hiroshima and Nagasaki bombs and the observation of persons exposed to atomic bomb irradiation in Japan was begun with the hypothesis already formed and with a reasonable expectation of a positive result. Despite this it has taken the surveillance of over two million person years in people exposed within 10 kilometres of the two hypo-centres to demonstrate convincingly the fact of an increased risk of leukæmia (Brill, Tomonaga & Heyssel, 1963). This study showed, in the period 1947 to 1958, an attack rate of 445 per million person years following exposure within 1·5 kilometres, compared with an attack rate of 34·7 following exposure at ranges between 1·5 and 10 kilometres. At the same time the attack rate in Japan as a whole was believed to be between 20 and 30 per million person years. During this time 82 cases were diagnosed in the population exposed at the shorter range compared with an expected value of approximately 6 or 7 if the attack rate at the greater ranges had prevailed.

The question of a leukæmogenic effect of diagnostic radiation also arose upon a background of prior knowledge of biological effects and the testing of the hypothesis by contingency methods is highly analagous with the work in Japan. In this case, however, the two main investigations with positive results have been retrospective, beginning with the supposed effect, leukæmia in childhood, and searching for differential previous frequencies of radiation both in affected children and in normal children matched in as many other relevant respects as was practicable. Stewart, Webb & Hewitt (1958) showed that 13·2 per cent of leukæmic children had received pre-natal irradiation compared with 7·2 per cent of controls. Ford, Paterson & Treuting (1959) found a radiation history in 26·9 per cent of leukæmias, 28·4 per cent of other childhood cancers, and 18·3 per cent of controls. Translated into relative risks both studies agree that the risk of leukæmia was doubled in irradiated fetuses compared with the normal population. In England and Wales about 6–7 per cent of childhood leukæmia can be said to have been caused by pre-natal radiation in the sense that the incidence would have been 6–7 per cent lower in the country as a whole if none had been irradiated. If these conclusions are accurate then medical applications of irradiation since the war have killed more people through leukæmia, in England and Wales alone, than have the atom bombs (from leukæmia) in Japan.

It should be mentioned however that not all studies of this problem have been unanimous. One particularly disquieting discrepancy is in a prospective study of irradiated pregnant women (Court-Brown *et al.*, 1960). Almost 40,000 live-born children of women who had pelvic or abdominal X-ray during pregnancy in the period 1945 to 1956, were investigated. The expected number of leukæmia deaths, supposing irradiation to have had no effect, was 10·5 and

the observed number 9. The conclusions of both the Stewart and the Ford studies would have led us to expect about 20. The paper refers also to other studies with equivocal or negative results.

A Portuguese study of patients who received a (radioactive) thorium based radio-diagnostic contrast medium has been reported recently (Da Silva *et al.*, 1965). A total of 2,377 patients so treated between 1930 and 1952 were identified and 1,107 traced; 699 were dead and 408 still alive. With so selected a population a set of controls could not be devised and there are other obvious difficulties with respect to the relative ease of tracing patients who are alive and those who have died. Altogether assessment of the traced population in relation to the general population was peculiarly difficult in this investigation. However, it was possible from national statistics to calculate expected specific mortalities based upon a series of extreme assumptions regarding bias, and the observed deaths from certain causes were shown, fairly convincingly, to be excessive. Deaths certified due to leukæmia, purpura or aplastic anæmia were clearly in excess, there was a high rate of cirrhosis of the liver, and 22 cases of hæmangioendothelioma of the liver were found, a condition considered by the authors to be "virtually thorotrast-specific."

From the point of view of method this study is a good deal less formal than the others so far mentioned and although the basic method is to demonstrate a statistical association the appeal of the conclusions arises in very large part from the well established carcinogenic effects of radioactivity, the fact that the raised mortalities are due to changes in tissues and organs which take up particulate foreign materials, and the specificity of one of the lesions which was found.

The relationship between thyroid cancer in young people (under 20 years) and previous irradiation of the thymus supplies the exception to the rule from the point of view of method. Simpson & Hempelmann (1957), the authors of the most important paper on this subject, acknowledge that Duffy & Fitzgerald (1950) made the initial observation. The latter authors, reviewing a group of 28 cases of thyroid cancer in childhood and adolescence remarked that 10 gave a history of therapeutic thymic irradiation in infancy. This was not the stated main objective of their study; they did not present any control frequencies for thymic irradiation and they felt unable to draw a cause/effect conclusion although they commented upon its possibility. Despite the absence of a formal control series their work implied that there was a statistical association and it does appear in this case that the hypothesis of causation arose from it. Simpson & Hempelmann (1957), in Rochester, N.Y., traced 1,502 patients who had been given thymic irradiation in infancy; they found 33 had subsequent tumours, 18 of them malignant including 10 thyroid cancers, and another 7, possibly 8, had leukæmia. Adequate control data were supplied and good dose-correlations were presented. The Rochester School has produced a series of excellent studies on this subject (Toyooka *et al.*, 1963; Toyooka, Pifer & Hempelmann, 1963; Pifer *et al.*, 1963), and Hempelmann has also written one of the more readable short accounts of the effect of therapeutic irradiation in general, using data available up to about 1960 (Hempelmann, 1961).

We can return now to the point made earlier, namely that it is something

of a challenge to have to cite instances where the method of numerical association has been an immediate source of a cause/effect hypothesis. Only the example of the thyroid carcinoma falls within this category. The main function of the method in the past has been to confirm and to quantify. This point is emphasized because of its importance at the present time. The circumstance which has made it important is the advent of the electronic digital computer in medical statistical studies and the consequent possibility of applying numerical association methods on a scale previously not practical. On the evidence of its past performance we may be disappointed if we expect the contingency method, automatically applied, to lead to valid causal inferences on a substantial scale.

### **Coronary Thrombosis**

The principle that the biological interpretation of a statistical interaction must be based upon external evidence also helps us to understand why in some situations, for example the relationship between carcinoma of the lung and smoking, we appear to have reached clear cut causal conclusions while in other situations, although there may be interactions in plenty, indecision reigns. The best current example of the latter situation is coronary thrombosis.

As with carcinoma of the lung the epidemiological study of coronary thrombosis in recent years began from the observation of an apparent increase in its frequency and the hypothesis that some recent change in the environment was responsible. In 1944, in England and Wales there were 5,610 deaths in males from carcinoma of lung and pleura, and in 1964 there were 15,027 deaths from cancer of bronchus and lung. With coronary thrombosis the position is less exact because the codings of causes of death have been changed. However, the increase has been studied critically by Record & Whitfield (1964) and it appears to be genuine and not an artefact arising from the changing age structure of the population or changing diagnostic abilities and fashions. The similarity of the two situations is such that it would seem to lead rationally to an investigation of the relationship between smoking and coronary thrombosis, and, if an interaction were found, to similar conclusions. In fact such studies have been carried out and the interaction has been found. What is more, as with carcinoma of the lung, different investigators are almost unanimous. The interaction has been found by Doyle *et al.* (1964) both in Framingham, Mass. and in Albany, N.Y., by Borhani, Hechter & Breslow (1963) in San Francisco, by Paul *et al.* (1963) in Chicago, by Doll & Hill (1956) in Great Britain, and by others. Additional references to this subject may be found in the report of the Surgeon General of the Public Health Service, U.S.A., entitled "Smoking and Health" (U.S. Department of Health, Education and Welfare, 1964).

Estimates of the relative risk of death from coronary disease according to the amount smoked vary, but for 20 or more cigarettes per day are generally between three times and five times the risk for non-smokers. For all smokers compared with non-smokers the ratio is about 1.4. Cigar and pipe smokers seem to resemble non-smokers more closely than cigarette smokers and this again is similar to the findings in carcinoma of the lung. To infer the exact



change in incidence which would follow specified changes in smoking habits is not a simple exercise and does not seem to have been accomplished but much of the increased incidence in recent years could probably be explained in these terms. The risk of deaths from coronary disease in smokers compared with that in non-smokers ( $\times 1.4$ ) is increased less than is the mortality from carcinoma of the lung ( $\times 10$ ). However, deaths from the first disease are much more frequent than deaths from the second, and the smaller *proportional* increase in deaths from coronary disease in smokers, represents a far greater *absolute* number than the number of lung carcinoma deaths attributable to smoking. Total mortality rates (all causes) are higher in smokers than in non-smokers; half of the excess is due to coronary thrombosis and only a quarter to carcinoma of the lung. At the other extreme of this scale carcinoma of the larynx has the highest relative risk ratio of all ( $\times 20$  between smokers and non-smokers in the U.S.A.), yet it accounts for only one per cent of the overall excess mortality in smokers.

From the public health point of view it is the absolute effect rather than the ratio of relative risks which is important, but from a purely biological point of view the relative risk ratio may hold more interest. In the case of primary respiratory tract carcinomas the relative risks in smokers and non-smokers are such that smoking, if on other grounds it is accepted as a cause, is virtually a necessary cause; the risk is therefore optional and indeed the disease can almost be defined in terms of smoking (compare soot carcinoma). With coronary thrombosis on the other hand, the risk is considerable even in the absence of smoking (higher than for lung carcinoma in the presence of smoking), and even though the pharmacology of smoking tends to confirm the interpretation that the association is causal, it is certainly neither a necessary nor a sufficient cause.

This conclusion, that smoking is only one of a cluster of causes, is confirmed by the long known association between hypertension and the risk of coronary thrombosis. Of course hypertension is not an environmental factor, although it may be partly determined by external causes. The predictive value of raised blood pressure with respect to coronary thrombosis has been widely confirmed, for example by Record & Whitfield (1964) in Birmingham, Kannel *et al.* (1961) in Framingham, Yano & Ueda (1963) in Hiroshima, Paul *et al.* (1963) in Chicago, Pell & D'Alonzo (1963) in Wilmington, Delaware, and by Borhani, Hechter & Breslow (1963) in San Francisco.

There is good evidence that the effects of hypertension and smoking are additive, and hypertensive smokers have about ten times the mortality of non-hypertensive non-smokers from coronary artery disease.

The cholesterol content of atheromatous plaques has led to analogous attempts to discriminate risk in terms of serum cholesterol and positive associations have been found which are additive with the hypertensive effect. For example Kannel *et al.* (1961) found a four-fold increase in risk passing from men with a serum cholesterol of 150 mg per 100 ml. to those with 300 mg per 100 ml. They found a similar four-fold increase passing from men with systolic blood pressures of 110 mm to men with systolic pressures of 180 mm. The combined gradient showed a 20-fold increase of risk passing from men with a serum cholesterol of 150 mg per 100 ml. *and*

a systolic pressure of 110 mm, to men with a serum cholesterol of 300 and a systolic pressure of 180.

Because only a minority of people have high levels of either serum cholesterol or blood pressure we find that these measures are excellent means of selecting small high risk groups. However, low cholesterol and low blood pressure do not adequately specify a very low risk and even in non-smokers we cannot reach the satisfactory position achieved with respect to carcinoma of the lung and say that a person without the high risk attributes is virtually safe from the disease. Therefore the search for additional predictive characters and measures (environmental or otherwise) and their combinations, has continued.

The cholesterol question has itself ramified in several directions. For example there have been experiments to devise methods of lowering the serum cholesterol, initially by controlling the amount of cholesterol consumed. But then it appeared that the best method was to arrange for a high dietary proportion of unsaturated fats (Joliffe, 1959). This raises the question whether a high cholesterol level can be considered causal at all, or whether it is to be regarded simply as an index of some other aspect of the diet. For example, one study showed that the wives of patients with coronary thrombosis had higher cholesterol levels than the wives of controls (Skyring *et al.*, 1963).

Several attempts have been made to measure the effect of cholesterol-lowering diets upon the risk of recurrence in patients who have had coronary thrombosis—mainly with negative results—but the crucial experiment in prevention does not yet seem to have been reported. Analyses have been made of the dietary fat content of different groups of individuals subject to different rates of coronary thrombosis and some interactions have been found, particularly with respect to international comparisons. However, Yudkin has reviewed this question critically and has demonstrated that these associations are erratic quantitatively and that a better prediction of national mortalities from coronary thrombosis is given by their intakes of sugar (Yudkin, 1957, 1963). In the general pursuit of dietary factors it can be seen that the contingency arguments have become detached, imperceptibly, from the cholesterol in the plaques. Methodologically speaking an improvement in the strength of a correlation or statistical interaction\* is a very dubious gain if the cost is the loss of a direct and plausible interpretation. This is a general problem in all multifactorial and multiple regression analyses. Indeed, formal multiple regression techniques have been applied to coronary thrombosis and the result was singularly unhelpful (Gertler *et al.*, 1959). In multiple regression analysis an equation is derived of the form

$$y = a + b_1x_1 + b_2x_2 + b_3x_3 \dots + b_nx_n,$$

where  $y$  is a numerically expressed dependant variable, in this case the risk of coronary thrombosis, where  $x_1x_2 \dots x_n$  are measured variables upon which  $y$  is thought to be dependent, and  $b_1b_2 \dots b_n$  are calculated numerical coefficients.

Of course the dietary hypothesis of the ætiology of coronary thrombosis

\* The term "correlation" is used customarily when the factors are continuous and quantitative, e.g. height, weight; the term "statistical interaction" is more general and includes also qualitative and discontinuous variables, e.g. sex, sickness.

has a base wider than cholesterol, and the rationale behind the general approach is well set out by Yudkin in the two papers referred to. Nevertheless its basis is diffuse rather than precise and it can be regarded as a direct consequence of this that the interpretation of the relevant statistical associations has produced results of a like nature.

The effect of obesity on the risk of coronary thrombosis is related to the dietary hypothesis and many investigations have been reported. In contrast with the popular medical belief that obesity is probably the most important factor of all, the results have been on the whole marginal and difficult to interpret. Keys & Fidanza (1960) supplied two series of patients from Minneapolis and Naples and, finding their results contradictory, reviewed a total of 18 major examinations of this question. Nine of these studies concluded that increased body weight was associated with an increased risk and nine concluded that it was not. Some of the disagreement may be based upon technical points, for example the exact definition of the condition being examined, and failure always to differentiate between increased body weight and obesity, but since this review several other large and competent investigations have produced negative, or at best marginal, results. Yano & Ueda (1963) reported negatively from Hiroshima. Paul *et al.* (1963) reported negatively with respect to height and weight in Chicago—despite some positive results with respect to somatotype and skin-fold thicknesses. Borhani, Hechter & Breslow (1963) reported marginal and irregular relationships in their San Francisco investigation. Pell & D'Alonzo (1963) in Wilmington, also found negatively on this question. The overall picture suggests that when obesity is associated with a high risk it is probably an indirect effect, the obese coming from a group whose risk is high for other reasons. If this interpretation is correct then deliberate reduction of body weight may not have a prophylactic effect.

The Framingham workers have shown that the more striking associations of coronary thrombosis apply also to cerebral thrombosis and the most valuable predictive criteria are abnormal electrocardiograms, hypertension, and smoking but not obesity (Kannel *et al.*, 1965). Morris *et al.* (see Morris, 1964, pp. 172–182) discovered that London bus drivers had larger waist band measurements than did conductors when their uniforms were issued and they had a higher rate of coronary thrombosis. Morris concludes correctly and cautiously simply that men who become drivers are different from men who become conductors. The problem of obesity is analogous with that of diet; it is difficult to see how the answer could be expressed in terms more exact than those of the question.

For this reason at least, the question of the influence of physical exercise with its manifest cardiac effects, upon the incidence of coronary disease is a more attractive interpretative proposition and currently it is being pursued with enthusiasm. Morris (1964) specifies the hypothesis well and examines it from several points of view. He regards the differences in incidence between London bus drivers and conductors as more probably due to exercise differentials than (primarily) to differences in body weight. He has quoted mortality rates in England and Wales for men engaged respectively in light, active, and heavy work, and a consistent gradient was shown to exist within each social class. Confirmatory evidence is quoted from Los Angeles, North

Dakota, Israel, Finland, and Poland. Since this short review was prepared other reports have appeared. Kahn (1963) reported differentials between different grades of American Post Office workers—postal clerks and letter carriers. Higgins, Cochrane & Thomas (1963) found differences between light and heavy workers in South Wales. Puchner *et al.* (1961) reported differences between different grades of railway employees in the North-western quarter of the United States, sedentary clerks and switch-men aged 40 to 59.

Thus, a considerable body of almost unanimous evidence on this point has been assembled. However, it should be noted that virtually the same technique has been used on each occasion and, unfortunately, it is subject to one particularly serious artefact. This arises from the likelihood that in a substantial proportion of men, early symptoms of the disease may lead to a change of employment before a specific diagnosis has occurred, and that such changes may occur differentially at different levels of physical activity. The onset of breathlessness in a postal clerk, a railway clerk, or a bus driver will cause less disturbance than in a postman, a railway switch-man, or the conductor of a double-decker. In the latter groups it is quite likely that some men with a high risk of being diagnosed, had they stayed in the job, will leave the industry or will arrange to be transferred to light work. Likewise, the ranks of the light workers may be swollen by breathless immigrants from other grades and industries. For the time being these difficulties of interpreting observational data in these so-called "survival populations" have not been adequately resolved and rigorous experimental approaches have not yet been reported.

### Developmental Studies

The applications of contingency methods so far discussed are of two main kinds. The first concerns the prediction, detection and interpretation of statistical interactions between two qualitative variables, for example thalidomide and malformation, or thorium and hemangioendothelioma. At a crude level almost all relationships between diseases and their causes can be conceived in these terms. However, scientific observations are of two kinds, acts of recognition and acts of measurement, and the second group of applications concerns the relationships between diseases and measured variables. I have touched upon obesity, blood pressure, and fat and sugar intake in relation to coronary thrombosis. Of course we recognize that many environmental and predictive measurements can be reduced to qualitative terms and conversely that taxonomies based on qualities can often be extended to quantitative terms.

However, we must now face the fact that a disease is itself a taxonomic group, and its definition sometimes quite arbitrary. Sometimes it is profitable to use an extended quantitative assessment rather than a crude division between normal and abnormal. There are occasions where this is the only sensible thing to do and a proper consideration of intelligence, growth, obesity, blood pressure, together with behavioural appraisals in general, would be impossible otherwise. Whenever the definition of the abnormal is not based upon an obvious bimodality it is difficult even to communicate its terms without reference to the distribution in the whole population.

When combinations of quantitative criteria must be considered there is scope for highly intricate statistical treatment (Baron & Fraser, 1965; Sneath, 1965), using multiple correlation techniques somewhat analagous to those of multiple regression.

Recent development of the quantitative approach has occurred especially in psychiatry, pædiatrics, and obstetrics with their special interests in developmental and behavioural patterns.

Since the war there have been two major studies in this country concerned with the continuing assessment of health and sickness in representative samples of children. The first, under the direction of J. W. B. Douglas is a study based upon a national sample of 13,687 children, all those born during one week in March 1946. The second, under the direction of F. J. W. Miller is a study based upon 1,142 school children, all those born during the month of May 1947 in Newcastle-upon-Tyne. The national sample has the advantages of size and assured representativeness for the whole country; moreover families were not lost when they moved from one area to another. The disadvantages in these respects of the Newcastle sample should be matched against its advantages of closer supervision, more frequent visiting, and a more uniform and more detailed level of investigation. Both studies have continued now through the school years and from each a number of reports have appeared or are in preparation (Population Investigation Committee, 1948; Douglas & Blomfield, 1958; Douglas, 1964; Spence *et al.*, 1954; Miller *et al.*, 1960).

Neither investigation was entirely a study of contingencies and both were concerned also with measurements of incidence, clinical description, and the derivation of frequency distributions, but contingency methods were extensively used. Many hundreds of statistical interactions were quoted in the texts of the reports, in tables, figures, and appendixes. The two investigations exhibited a parallel evolution in the pattern of usage, seemingly dictated by a natural re-orientation of interest. These changes may indicate the direction of future developments.

The initial purposes of the national survey were to collect more "detailed information on social and economic aspects of child bearing". However, it supplied an entry to studies of biological subjects, the incidence and associations of prematurity, the survival and development of premature infants, patterns of infant feeding, and others. Later, on the basis of a sub-sample, information was collected on respiratory tract infections, infectious diseases, accidents, and the different kinds of fatal illness up to the age of five years. The Newcastle study was concerned with episodes of sickness from the beginning, and their study was its primary object.

It is not possible here to review these studies comprehensively. However, a few examples will suffice to show that in their early stages they tended to present statistical associations between simple qualitative variables and that these associations were capable of, and probably intended to provoke, fairly direct interpretation. For example, both studies showed that the presence of a school child in the house was associated with an increased risk of pre-school children contracting common infectious fevers and the obvious interpretation is in terms of transmission and contact. Both studies showed that the frequency of accidental injury in early life was correlated with the

standard of maternal care, with higher frequencies where the standard was poor. The Newcastle study also showed that accidents were more frequent in over-crowded conditions, but this was true for non-domestic as well as domestic accidents, and poor mothers tended to live in over-crowded conditions. It seems likely that a high risk of accidents is determined more by the type of family, its attitudes, and the degree of deliberate protection given to the children, rather than over-crowding. This is not to say that the amenities play no part in accidents; obviously they must. It is impossible to fall downstairs if there are no stairs or to get burned at the fire if there is no fire. However, these demonstrations were apparently intended to be interpreted very directly in terms of maternal incompetence, and such interpretation seems reasonable.

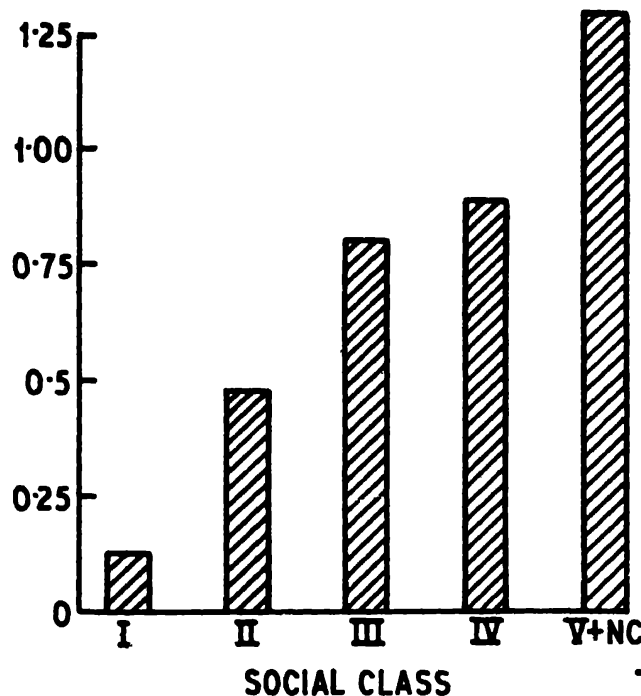


FIG. 12.1. Bronchitis and Pneumonia in Childhood. Mean number of attacks per child in first five years of life. (From "Growing up in Newcastle-upon-Tyne", Oxford University Press, 1960, by courtesy of the Nuffield Foundation.)

In both investigations, however, the possibility of a direct conclusion of this kind is exceptional, and a more usual pattern of presentation is a battery of associations between a particular type of disease episode and a cluster of biological and social indices. Douglas & Blomfield (1958) showed that the frequency of lower respiratory tract infections in the first two years of life was higher in the children of manual workers than of non-manual workers and higher when the housing was poor and the standard of maternal care low. The Newcastle workers confirmed the social class gradient, and the association with poor maternal care, demonstrated that severe respiratory disease was more frequent in small houses than in large ones and was highly correlated with a battery of tests of deficient physical care. Respiratory illness was also more frequent in those districts of the city where the density

of population was highest. All these effects were particularly clear with the relatively severe illnesses classed as bronchitis and pneumonia, and less clear or absent in the milder illnesses classed as severe colds. A general interpretation advanced by the Newcastle workers was that these associations represent an effect of social conditions upon the severity of respiratory disease rather than upon its overall incidence. This general conclusion has been confirmed by other studies; steep social correlations have been found for fatal respiratory illness and for pneumonia, and investigations employing relatively crude criteria such as these have experienced little difficulty in demonstrating social correlations (see Fig. 12.1). On the other hand more intensive investigations of respiratory illness in childhood, seeking to record every illness and not just the severe ones, have not been able to achieve this. The overall picture is curiously paradoxical; the greater the effort in searching for them, the less likely are the social correlations of respiratory illness to be demonstrated.

It should be evident from this how difficult it would be to demonstrate specific hypotheses, such as have been advanced from time to time, that the essential determinants of severe respiratory illness in childhood are cold bedrooms, smoke pollution, high humidity, or protein or vitamin deficiency. There is nothing in the information presented which would contradict any of these hypotheses and yet none which would specifically support them. More important, attempts to find a statistical association between the frequency of severe respiratory disease and any of these variables, would almost certainly succeed if the investigation were sufficiently large and competently done; such a demonstration would not depend upon, nor would it demonstrate, the truth of such specific causal hypotheses.

The Newcastle investigators used the multiple, or battery, approach extensively and a number of conditions showed strong and multiple correlations with a large group of adverse social factors, themselves highly inter-correlated. Indeed, apart from respiratory disease, they grouped together alimentary infections, accidents, enuresis, and retarded growth as "diseases of the social environment". In these groups of illnesses at least they have turned away from direct mechanistic interpretation of statistical associations and used them rather to extend the concept and description of the disease.

There are some who give little for this approach, regretting the apparent retreat from precise interpretation, and of course it is to be regretted when precision is not possible. However, it seems even more important that when it is not possible this should be recognized, and that inferences of this kind should be seen to be outside the domain of simple statistical demonstrations.

Up to this point the two studies were concerned mainly with short term events and episodes but as the children grew older the age of acute illness receded and disability emerged. The national study presented accounts of growth failure, bed-wetting and delayed social milestones, and their relationships with prematurity. The Newcastle study also gave accounts of strabismus, convulsions, speech disorders, behaviour disturbances and social mal-adaptation together with an account of the emergence of recurrent and chronic respiratory disease patterns. In addition there were the residual disabilities of poliomyelitis, congenital malformations, accidents and other acute episodes. Finally the latest analyses in both studies have become pre-

occupied with intelligence, social adaptation, emotional development, and the reasons for their breakdown.

In order to examine these last aspects successfully it has been necessary to see disability as a tail of a distribution affecting the whole population. Studies of the genesis of growth failure and mental retardation in relation to their medical and social backgrounds are more comprehensible if the whole pattern of distribution of development is studied. There are some children with Down's syndrome (mongolism) or microcephaly, for example, where mental defect presents as a relatively precise syndrome, but the majority of subnormal children present no clear criteria of demarcation from the rest of the population. The only sensible way of studying the genetic and social determinants of disability here is in the context of the study of development throughout the population as a whole. The emergence of respiratory cripples, of the deaf, and of non-communicating children, and their relationship to environmental determinants, likewise requires that the whole range of function is studied.

This continuing metamorphosis from the study of episodes, to the study of disabilities, to the study of the whole range of function has resulted in further changes in the interpretation of statistical interactions with environmental factors. This has been in the direction of progressive integration. We have already seen how the "diseases of the social environment" enforced a graduation from the "A causes B" interpretation to the position where environmental associations are regarded as part of a complex of descriptive attributes. When a whole range of function is considered, for example intelligence, this integration is carried a stage further and indeed it is difficult to use the term "function" at all. The operative concept of the national and Newcastle studies in their later stages is that of "performance" which may be defined as function in relation to a specified environment. The environmental interactions of intelligence amplify the definition and specify the level of performance in a wholly integral manner.

The parallel pattern of development in these two studies cannot be relied upon to indicate accurately the way in which the general pattern of interpretation of contingencies may go. However, enough was said earlier to show that there are at least very serious doubts over the supposed logic of statistical decision and statistical inference procedures. The relatively informal approaches of these two investigations, recognizing frankly the ultimate logical intractability of the statistical decision-making problem, may indicate the future movement of events and thinking in this field.

### **AUTOMATIC INFERENCE**

The structure of this chapter, so far, has been based essentially on method and the problem of interpretation, and it is intended to reflect a contemporary re-appraisal of statistical inference procedures. The controversy surrounding this subject springs chiefly from logical considerations but its urgency has been heightened by the advent of computer methods. The computer has made statistical computation easier and it has made possible the compilation of contingency tables on a scale not previously practicable. The proposal to "test everything against everything" now seems to be within the bounds of possibility.



There are two steps involved in this approach; first the compilation of the contingency tables and second, the computation of tests on which it is proposed a decision can be based. The first step involves the currently developing techniques of record linkage; they are popularly regarded as complicated and difficult but in principle are very simple. The second step is largely identified with statistical significance tests, popularly believed to be established and well founded procedures leading to reasonably clear decisions, but in fact of doubtful utility and widely misunderstood and misused.

### **Record Linkage by Computer**

Despite the recent interest, record linkage turns out to be a rather ancient procedure, something we have all done, even if we did not give it so imposing a title. However, the advent of mechanical and electronic methods has had the effect, apart from offering prospects of achieving the previously impracticable, of requiring an exact analysis of the logical steps of the process. Whether the process is carried out by hand, by filing card, by punch card or by computer there are three basic stages, namely (1) identification, (2) sorting, and (3) matching.

Ideally, identification of individuals (i.e. records) should be on a numerical basis since each patient can have a unique and unambiguous number. Furthermore such numbers exist, for example in the United Kingdom, the National Health Registration Number. However, Smith (1963) has examined the practical difficulties and they are formidable. The National Health Registration Number itself may vary in format depending on when it was issued, whether the person lived in a large institution at the time, or whether he was in the Armed Forces. Furthermore, few people know or can easily produce their number and the records to which it is attached are protected from inspection by legislation. Its chief purpose is the calculation of General Practitioner remuneration and the avoidance of duplicate payment, with respect to which it is said to be partially successful. It is of course irrelevant outside the United Kingdom and in any case the number may not be attached to all of a group of files which it is necessary to link.

For these reasons most workers prefer to use a non-unique identification derived from data which individuals can usually supply, particularly their surname, their given names and their date of birth. Disadvantages are that no particular identifications can be guaranteed unique and false matches may be made. Conversely, variable and illegible spelling of surnames, variable versions of given names, and the surprising inability of many people to give the same birth date twice, may prevent true matches from being made. However, it seems possible with sufficient ingenuity of coding to achieve an acceptably high proportion of true matches at the cost of an acceptably small proportion of false matches. The definition of acceptability depends in each case on the cost, the purpose, and the consequences of error. In a large investigation linking drug prescriptions with subsequent maternities we might be relatively unconcerned if we lost 10 per cent of true matches, but if we were processing the files of a Blood Transfusion Department or a register of drug hypersensitivities, error rates many times smaller than this might still be unacceptable.

One particularly troublesome item is change of name on marriage and this is a serious hindrance in studies of teratogenic drugs where, in a sizeable proportion of cases, the change has occurred between the time the drug was prescribed, in early pregnancy, and the delivery of the child. The setting up of contingency tables is not the sole purpose of computer linkage methods and the compilation of pedigrees from registrations of marriage and birth is receiving enthusiastic attention; the problem of the maiden name arises here also. For these reasons the inclusion of the mother's maiden names on birth certificates and other records is desirable and now widely advocated (Newcombe, 1965; Acheson, 1965). Sorting, in the computing sense, is simply the arrangement of records in descending or ascending order with respect to an alphabetical or numerical value, or combination of values, contained in the record. Frequently it will be required after some kind of mixing procedure, for example the addition of two files from different sources, and the basis of the sorting will be some numerical coding of the name and birth date. Following the sorting procedure records with similar names and dates will be found adjacent to each other.

There are several general methods of sorting and much programming effort has gone into finding the most economical of these in terms of machine time. On a punch card sorting machine the method is very simple; the pack is sorted on the least significant digit first, the sub-packs stacked one upon the other and the pack re-sorted on the next digit . . . and so on. On a computer the technique for a large file depends upon the devices available. If magnetic tapes are being used then the more tape-decks (sub-packs) there are the faster the job is done. Random access stores, such as magnetic storage discs with multiple tracks, where the computer does not have to spend time winding backwards and forwards on long stretches of tape, are a particularly useful facility.

The final step of record linkage procedures is matching and the procedure is simply to scan the file from one end to the other comparing each record with adjacent records. If they are identical a probable match is recorded and if they differ by some arbitrary value a probable non-match is recorded. Obviously it is possible, following close matches of various kinds, to conduct additional tests and to make decisions accordingly.

### Significance Tests

When we argue from a population to a sample the reasoning is deductive and forward looking, from the general to the particular, and it can be based upon abstract probability concepts analogous with heads and tails, dice, etc. If the mortality of a disease is 0.3 (30 per cent), then the survival rate is 0.7 (70 per cent), and the probability of the next six cases dying is  $0.3^6$ . The probability of five out of the next six dying is  $6 \times 0.3^5 \times 0.7$ , . . . and so on by relatively simple algebraic rules, according to the successive terms of the expanded binomial  $(p+q)^n$  . . . where  $p$  is mortality and  $q = 1-p$ .

Backward looking arguments are not so simple. As an example let us attempt to differentiate between two diagnoses on the basis of a clinical finding (F) in a given individual. We are told:

1. Disease A results in finding F on 20 per cent of occasions.
2. Disease B results in finding F on 10 per cent of occasions.

3. Disease B is three times as common as disease A.
4. No other condition results in finding F.

If we are given the presence of A *or* B but do not know which, then the probability of A is 0.25 and of B, 0.75. *If A then* the probability of F is 0.2, therefore the probability of A *and* F together, among all individuals with A *or* B, is  $0.25 \times 0.2 = 0.05$ . *If B then* the probability of F is 0.1 and by similar reasoning the probability of B *and* F is  $0.75 \times 0.1 = 0.075$ . Therefore, given F,

$$\begin{aligned} \text{the probability of A is } & 0.05/(0.05 + 0.075) = 0.4 \\ \text{and the probability of B is } & 0.075/(0.05 + 0.075) = 0.6 \end{aligned}$$

This is known as a Bayesian argument after the Rev. Thomas Bayes to whom is attributed the first analysis of its logic. Note particularly the minimum information necessary for deciding the most likely cause of the effect: (1) the frequency of F for each cause, (2) the relative probabilities of all possible causes. We know that common diseases are commonly diagnosed and it seems likely that medical diagnosis employs processes analogous with the Rev. Thomas's logic (see Ledley & Lusted, 1959). This is discussed in more detail in its relation to computer-assisted diagnosis in Chapter 1.

There is an analogy with this process in the situation where we observe a statistical correlation between two variables in a sample and have to decide whether this interaction represents only a sampling variation with no interaction in the general population (diagnosis 1) or whether it represents a general relationship requiring a biological or other explanation (diagnosis 2). The hypothesis that there is no interaction in the general population is called the Null hypothesis. The now customary approach is to apply a significance test, for example  $\chi^2$ , and through this to estimate a value *P*, which is a statement of how often the finding—or a more extreme finding—would occur in such a sample if the first diagnosis were correct.

Sometimes, although it is not often practicable, the *second* diagnosis may also be specified with sufficient precision to calculate how often the interaction would turn up in such a sample if that diagnosis were true.

However it is never possible to state the relative frequencies or probabilities of the two diagnoses before the experiment began. Consequently the full Bayesian argument is not possible and the logic incomplete. Therefore a significance test is not a sufficient validification of a particular biological interpretation and a logical decision cannot be made upon such evidence alone.

If *P* is very small we may if we wish reject the null hypothesis as unreasonable, *provided that the alternatives can be specified and are themselves reasonable*. This expression (in italics) cannot be quantified, and we must not entertain prospects of automatic inference by this route.

The value *P* comments upon the consequence of the null hypothesis but says nothing whatsoever about the consequences of any of the alternative hypotheses, nor about their relative prior plausibilities. Therefore, and we must be quite specific about this, *P* does *not* tell us the probability of the null hypothesis (or any other hypothesis) being true or false, and it does *not* tell what is our chance of being right or wrong if we decide one way or the other.

$P$  is best regarded as an approximate quantification of only one of the elements which would be necessary for making a fully logical decision.

It may seem unnecessary to labour these points but the whole subject has been very widely misunderstood. Errors occur frequently in well regarded texts. For example, Bradford Hill (1946) crystallizes precisely the illegal logical leap from a deductive to an inductive mode of thought in the words "... 'significance' i.e. unlikely to have arisen by chance". A correct statement would be "unlikely to arise by chance if the null hypothesis were true", that is a forward looking deductive argument, but this is very different from the backward looking "unlikely to have arisen by chance". Moroney (1951) equates " $P = 0.05$ " with "this result could arise by chance once in twenty trials". Does he mean "will arise" or does he mean "will have arisen"? Clearly the first is correct and this is probably what he means but later he invokes "the principle that what is inherently unlikely, is not likely to have happened". Unfortunately this is not true; unlikely events are happening all the time. The conclusion that an inherently unlikely event is unlikely to have happened depends on what the alternatives are. Provided that there *are* alternatives we may perhaps agree that that which is *less* likely to happen is *less* likely to have happened but we are not entitled to say, without qualification, that it is *unlikely* to have happened.

The basic logical error in both texts is an unwarranted leap from a conditional statement ... "*if* the null hypothesis were true *then* ...", ... to an unconditional one, unwarranted because it omits the step of declaring the manner, stating the probability and deducing the consequences of the null hypothesis not being true.

It must be said that some authors specifically reject the Bayesian approach to statistical inference (Fisher, 1935), but claim that valid and useful systems of inference can be constructed without recourse to it (Kendal, 1948). It is not possible to go into these arguments here but Hogben (1957) has reviewed the field from an historical viewpoint and in a forthrightly destructive way, while Wrighton (1967) has demonstrated the absurdities in the fundamental terms of information theory.

### NON-CONTINGENCY METHODS

The American report, "Smoking and Health" (Chapter 3) presents a short essay on the criteria of the "epidemiological" method. Five main criteria are set out and all refer to the interpretation of statistical associations. The interpretation of associations is highly identified with epidemiology in a way suggesting that this is the whole of epidemiology. The context perhaps excuses this restricted outlook—it is of course an excellent report and likely to be regarded as a classic—but it reflects a very widespread point of view. The main point made in the earlier parts of this chapter is that although contingency methods can be used in the development and testing of highly specific hypotheses, the actual interpretation is informal rather than logical and never specific. Consequently the contingency method is on all fours with other epidemiological techniques usually (and correctly) regarded as non-specific.

These other approaches are mainly concerned with aggregations of events in time, in space, in time and space jointly, in families and in other

social groups. They are concerned first with the detection of the aggregations and second with an analysis of detail, and a review of the main recent lines of advance will now be presented.

### **Analyses of Frequency Distributions**

The form of a frequency distribution has seldom proved to be of much use in the detection of environmental causes of disease and the main purpose in mentioning it is simply to say so. Genetic variations have sometimes been investigated successfully, and the bi-modal distribution of the minimum concentration at which PTC can be tasted, is a good example. However, the peaks of two identical normal distributions must be separated by more than two standard deviations before the distribution of their sum even begins to be bi-modal. If the two distributions are of unequal height a wider separation is necessary. Consequently, where we are dealing with minority groups, as we are with most diseases, we must not often expect successful discrimination on this basis. Moreover, the form of a frequency distribution is sensitive to such artefacts as number preference and sampling variation, so that bi-modality, when it is found, cannot be relied upon. These facts have undoubtedly contributed to the sterility and inconclusiveness of much recent discussion on the genetic definition of essential hypertension.

One recent study of hæmatological data (Elwood, 1964) in both men and women in Belfast did succeed in making a useful contribution to our knowledge of anæmia. By comparing the tails of both distributions it was possible to reach some tentative conclusions concerning the proportion of women anæmic through menstrual loss.

Knox & Walker (1957) studied the frequency distribution according to birth rank of infants first affected in their family with rhesus hæmolytic disease of the newborn. They were able to compare their observations with the predictions of two distinct hypotheses. The first was that the risk of rhesus sensitization was determined by genetic factors external to the rhesus blood group system and the second that it was due to an adventitious factor operating at one of the pregnancies. The first hypothesis would have produced a shift to the left of the expected birth rank distribution because the determining factor would have been operating from the beginning of the sibship. The facts fitted the second prediction well and did not fit the first prediction, and the second hypothesis was preferred.

However, relatively precise applications of this kind are rare and opportunities for exploiting them do not seem often to arise.

### **Distributions in Time**

When events are distributed in time in some systematic manner it is difficult to propose a genetic cause for the pattern. Slow long term trends and changes associated with migration or other major alterations of population structure are exceptions, but the majority of time effects must be attributed to environmental causes. Consequently the demonstration of systematic time patterns has been extensively applied to diseases where the balance of nature and nurture is uncertain. Less frequently, the specific nature of an environmental determinant has been explored with this technique.

There are many ways in which a time distribution can depart from random

but the three patterns most relevant to the demonstration of environmental influences are trends, cycles, and clumps.

### **Trends**

Trends, not unnaturally, attract more attention when the incidence increases, than when it decreases. The decreasing incidences of intestinal carcinoma, tuberculosis, and death from measles or whooping cough do not occupy a central position in the development of our understanding of their ætiologies. Contrast this with the increasing death rates from coronary thrombosis, carcinoma of the respiratory tract, leukæmia, and traffic accidents. This is not only a consequence of alarm; it is easier to identify and to take an interest in causes which are becoming more obtrusive than in those which are disappearing. It would now be a very difficult proposition to investigate the causes of the decline of chlorosis, or even to establish its existence as a useful taxonomic entity.

Of course, causal associations can never be inferred simply from parallel secular changes but it may be difficult to infer them without demonstrating this feature. Moreover a detailed analysis of a trend, with attention to detail, may provide valuable clues.

Leukæmia is a good example. Court-Brown & Doll (1961) studied the secular changes in deaths from leukæmia in the years between 1911 and 1959. Not only did they demonstrate a rate of increase during this period of between 4 and 5 per cent per annum, but they also showed that the rate of increase differed at different ages. An alternative way of putting this is to say that the age distribution, as well as the overall incidence, changed. Current figures show that there is a peak in incidence at about age 3; this peak appeared for the first time around 1920, was not present before that time, and has persisted ever since. Lee (1961) has also shown the presence of an additional age peak at about 15 years of age; until he carried out the requisite detail of analysis this peak had been hidden by the division between the conventional age groupings. This peak appears to be specific to the post-war period. Doll (1965) has written a good review of these changes and of the succession of appearance of different age group peaks in different countries. The causes of these changes have not yet been identified but at least we can infer that there has been a general increase in the incidence of leukæmia, and that this must have arisen from several distinct sets of circumstances operating in different age groups, appearing at different times and in successions which differ in different parts of the world.

Billington (1960a, 1960b) has made a study of changes in the age and sex distribution of gastric ulcer in Australia. From about 1943, fairly abruptly, there was an increase in the incidence of gastric ulcer in women of child-bearing years. This has resulted in a shift to the left in the age distribution of affected women, and a change in the sex ratio of affected young adults. So far it has not been accompanied by changes in any other country examined but it recalls the situation in Victorian England. Gastric ulcer, especially bleeding gastric ulcer, was then characteristically a disease of young women and was attributed to stays and tight-lacing. Billington claims to have excluded this theory in Australia at the present time. Admittedly it is not certain that the gastric ulcer of Victorian England was the same disease as

that in present-day Australia. Billington finds that the Australian cases are chronic ulcers but has not been able to find sufficient evidence to distinguish between acute and chronic ulcers in Victorian England. However, the question does arise from these studies what factor could conceivably be common to both situations. Gin has been suggested!

### **Annual Cycles**

It is seldom possible to be certain that a demonstrated trend is not part of a slow cycle; trends in the mortality from anencephalus over periods of many years have been shown to change their direction from time to time. However, it is seldom that we can make a specific interpretation from a long-term oscillation, and the cycles which offer the best prospects are those which correspond with natural cycles in the environment.

The annual cycle is one of these and in recent years the most interesting have been those discovered in leukæmia, other malignant diseases, and certain congenital malformations.

Lee & Gardner (1965) have recently reviewed the evidence with respect to malignant disease. Much of it is based upon the National Cancer Registration Scheme of England and Wales and an annual cycle was demonstrated based upon the date of the first reported symptoms. Leukæmia was the first disease to be examined and there appeared to be a characteristic seasonal pattern with two peaks, one in January and one in June. Over the age of 45 the winter peak was larger than the summer one, at ages 20 to 44 they were about the same size, but under the age of 20 the summer peak predominated. Knox (1964) collected a second series of data independently of the National Cancer Registration Scheme and this seemed to show the same pattern of a summer peak in children. Furthermore, Lee & Gardner (1965) collected data from Australia and New Zealand which also showed a summer peak (in December).

At this stage it looked as if this was a specific feature of leukæmia and that the pattern might be relevant to a supposed infective precipitation, but Lee has extended this type of study to other tumours in the National Cancer Registration Scheme and has found similar patterns here. He suggests that for some reason unknown there may be a general increase in the activity of malignant tissue of all kinds in the early summer.

Annual cycles have been demonstrated in three types of malformations. First they have been demonstrated in malformations of the nervous system, particularly anencephalus and spina bifida (Guthkelch, 1962; McKeown & Record, 1951; Record, 1961). There is an excessive winter and spring incidence both in England and Wales and in Scotland, although strangely enough, not in the United States (MacMahon, Pugh & Ingalls, 1953). Dislocation of the hip has been shown to be more frequent in winter births (Record & Edwards, 1958) and patent ductus arteriosus is more frequent in summer births (Record & McKeown, 1953). It is a curious fact, not easily explained, that the three malformations with demonstrable seasonal variations are also those three which, in contrast with almost all other malformations, have more females affected than males. In the case of patent ductus, it is possible that the seasonal variation is limited to the females (Record & McKeown, 1953).

Specific interpretation of one of these seasonal patterns has been attempted. It was suggested that the seasonal pattern of hip dislocation was attributed to cold weather, and it was proposed that heavily clothed and covered infants were held in extension whereas in the summer they were permitted to flex their hips. Racial differences, for example, between Africans and Lapps, were also consistent with this. However, there is also evidence of seasonal variation in hormone output in pregnant women and this may affect the laxity of the infants' hips. The seasonal variation of the nervous system malformations has not been interpreted exactly. While it is difficult to avoid the conclusion that there is an environmental cause for the seasonal variation, it does not necessarily mean that there is an environmental cause for the malformation. It is possible that there is a seasonal variation in the risk of abortion or resorption of foetuses with this malformation, and that the effect demonstrable in infants born after 28 weeks arises because we are dealing with a survival population. However, this disease also has an extremely steep social class differential and considerable urban-rural and wider regional variations in incidence, and these facts combine into a convincing argument for the existence of major environmental determinants. At this moment their nature is not indicated and there is little evidence on which we can begin to distinguish between infective, toxic and deficiency factors.

The seasonal variation of patent ductus could also, feasibly, be due to a post-natal survival factor since this disease is frequently not diagnosed at birth.

### Shorter Cycles

Between the annual cycle and the weekly cycle there are no very well marked oscillations in the general environment. I know of no very convincing variations according to lunar cycle and if there were it would be difficult to explain them in simple environmental terms. However, deaths from homicide show an interesting pattern. For many years the Registrar General has published figures of deaths by cause and by month and these data can be used to investigate cycles occurring in fractions of a year. In the ten years 1954-1963 there were 18 per cent more deaths from homicide in the first month of each quarter, compared with the other two months. This *might* be the Mafia redeeming uncollected debts quarterly; it is more likely to be an artefact arising from law sittings, coroners courts, and delays in diagnosis, although the Registrar General *says* that these are the months of death and not the months of registration.

In contrast with the difficulties of interpreting annual cycles, the causes of weekly cycles have usually been so obvious that it is difficult to think of instances which have contributed substantially to our understanding of environmental causes. Different types of accidents, especially road accidents, vary according to the day of week (Commissioner of Police of the Metropolis, 1963) and absence from work through sickness also varies by day of week. The most obvious explanation of the latter is the effect of Monday morning but this may not be only a question of the interpretation which sick people put upon their symptoms on different days. There is some evidence that the frequency of respiratory disease does vary according to the day of the week. Clearly there are weekly variations in patterns of human contact and



the opportunities for transmission between families and within families. There are also weekly variations in the density of atmospheric pollution. Byssinosis is an example of a specific industrial air pollution which was discovered because of its cyclical pattern. Sensitivity to fine cotton or jute dust produces a febrile reaction on Monday mornings, soon relieved by desensitization, but recurring again after a week-end's freedom from exposure.

This has been known for many years and weekly cycles have not often suggested a new departure in thinking, but there is one, recently demonstrated, which does.

This is the demonstration (Cameron & Asher, 1965) that unexpected deaths in infants ("cot deaths") are more frequent at weekends than during the rest of the week. It is possible that there is an artefactual element here. It may be that doctors are called less readily or are more difficult to call at weekends and that this affects the proportion of rapidly progressive infantile illnesses which come to the notice of the coroner. However, the cycle is a well marked one and is probably not explicable, at least not entirely, in this way. For similar reasons weekly cycles in infection rates do not seem capable of producing such a result and we are left with the strong suspicion that many of these deaths must be due to some factor in infant care which varies according to the time of the week. A recent report on this subject (Ministry of Health, 1965), found a high incidence of soft pillow, shared bed, bottle feeding in the first two weeks of life and other possibly asphyxiating circumstances in children who had died in this way but it is difficult to suppose that these might vary according to day of week. These children did have a high rate of respiratory symptoms in the few days before death and as we have said this may explain some of the weekly variation. However, it probably does not explain all of it and we must now seriously consider the possibility that some unsuspected element in child care technique is both harmful and has a weekly cycle. We shall have to consider possible toxic effects of sedatives, aspirin and other commonly available drugs, alcoholic beverages, purgatives, and the alcoholic carminitives so often given to infants.

Cyclical variations according to time of day have not been widely used, possibly because they are applicable only to diseases of comparatively sudden onset. It was demonstrated some years ago that numbers of births vary according to time of day (Charles, 1953), and so does perinatal mortality, but these are simple observations without specific interpretations. More recently acute intussusception of infants has been shown to vary by hour of day according to the time of the first symptom (Knox, Court & Gardner, 1962; Gardner *et al.*, 1962). The great majority of onsets were between 5 a.m. and 5 p.m. In combination with several other demonstrations, including the frequent presence of adenovirus infection in affected children, and differences in feeding techniques between affected and unaffected children, this observation helped to establish the likelihood that the origins of intussusception are explicable in mechanical and hydrodynamic terms. It appears that a combination of partial occlusion of the intestinal lumen by enlarged lymph nodes together with a requisite degree of intestinal activity, possibly expressible in terms of rate of fluid flow, are both usually necessary, but neither is sufficient.

### Time Clusters

Large scale non-cyclical fluctuations in the incidence of disease are highly identified with infective processes and the basic ætiology of the majority of the diseases exhibiting this feature is fairly well known. Not all epidemic diseases are infective and in recent years epidemics of toxic polyneuritis from food adulterants, notably triorthocresyl phosphate, have been reported. Another toxic epidemic of unusual interest was reported by Clements (1958, 1960) from Tasmania. In 1956 there was a sharp epidemic in the channel ports of Tasmania of goitre in children. This has been attributed to a government free milk scheme for children, beginning in 1950, which by altering the market for and profitability of milk resulted in a change of cattle fodder practice. It appears that there are goitrogens or their precursors in kales and in certain weeds infesting new pasture land, and that they are excreted in milk.

With respect to infective epidemics there has been a recent revival of an interest prevalent some 50 years ago. Essentially this line of enquiry sets up stylized mathematical models of epidemic behaviour and attempts to match results with observations. Parameters such as susceptibility, immunity, infectivity, and so on can be varied in an attempt to find a good match. The recrudescence of interest is chiefly attributable to the possibility of using computer methods in order to run models experimentally and to explore situations where the mathematics are difficult or intractable (Kendall, 1965). The purely mathematical approach usually necessitates excessive stylizing and simplification of the situation and the computer approach is less restricted in this respect but so far little seems to have been added to our understanding of the biology of these processes.

A more promising approach to the study of irregular epidemicity is the study of small-scale clusters. Miller *et al.* (1960) in their study of Newcastle children observed two attacks of acute glomerulonephritis in five years and both attacks occurred within a few days of each other. We know enough about nephritogenic streptococci to suppose that this was probably not a coincidence. The particular problem here is whether there is a connection between events known to behave in an epidemic pattern. A more important problem is to detect epidemicity in a disease whose epidemic potentials are not known. This question arises with many classes of sparsely occurring events and has risen recently with respect to malformations and malignant diseases.

An example is the malformation tracheo-oesophageal fistula and/or oesophageal atresia. Knox (1959) has reported two series in England, Babbott & Ingalls (1962) have reported one series in the U.S.A., and Kucera (1966) has reported a series from Czechoslovakia in which the pattern of events appeared to be non-random. The problem was not so much in detecting clusters, which was all too easy, but in knowing whether or not they were genuine.

Intervals between random events are distributed in a manner with which we are intuitively unfamiliar. The shorter they are the more frequent they are and this is unlike most of the frequency distributions with which we deal intuitively every day. With heights, weights, I.Q., and so on, we expect that a measurement which is less than the average occurs much less frequently

than does an average measurement. Probably it is a carry-over of our intuitive data processing from these more familiar circumstances to the less familiar world of intervals between events, which is responsible for our capacity to see events "coming in threes", and eventually to ignore these occurrences as one of the inexplicable facts of life.

However, a few useful methods of statistical analysis have now been developed and although none of them can be regarded as universally applicable each of them can be applied with some circumspection in different circumstances, and when several sets of data have been assembled as with tracheo-oesophageal fistula, a diagnosis of epidemicity becomes possible. It goes without saying that if a cluster can be associated in time with some plausible precipitating event then the argument is much stronger, especially if the association is repeated in different sets of data. Following the examples of rubella and thalidomide attempts of this kind have been made (Leck, 1963; Coffey & Jessop, 1959, 1963; Stoller & Collmann, 1965) especially with respect to malformations, but so far no demonstrations additional to these two have been convincingly made.

### **Geographical Distributions**

Problems of interpreting distributions of events in geographical terms are analogous with those just discussed. There is no exact geographical analogue of cyclical changes but there are analogues both of trends and of clusters. Hewitt has published two different examples of geographical gradients, the analogue of the trend. The first (Hewitt, 1955) examined mortalities from leukæmia in England and Wales and showed a diminution corresponding with a progression from south-east to north-west. More recently Hewitt (1963) has studied spina bifida in the United States, demonstrating a high incidence on the eastern seaboard and a progressive diminution towards the west and south-west of the country. Presentations of this kind are essentially visual and it is difficult to formalize their logic. Howe (1963) has published a large series of maps of England, Wales, Scotland and Northern Ireland setting out shading densities corresponding with standardized mortality ratios for several diseases. An example is given in Fig. 12.2. When interpreting maps such as these allowance must be made for the fact that the amount of shading depends as much upon the geographical area of a region as it does upon the incidence of the disease among its inhabitants and that the number of inhabitants is not necessarily proportional to the area. For example, the distribution of tuberculosis deaths in females shows three small black spots representing Middlesbrough, Sunderland and Gateshead, yet they represent more cases of disease than the large black areas of Inverness and Argyll. Nevertheless patterns emerge which are compatible with our understanding of the ætiology of some diseases, for example the dark spots on a light background corresponding with urban concentrations of respiratory carcinoma. The regional variations of mortality from carcinoma of the stomach, also well known from earlier studies, are shown to persist, with concentrations in the west and north especially in North Wales. The curious concentrations of deaths from peptic ulcer in London, Birmingham, Hereford and parts of Scotland supply food for thought.

Localized geographical concentrations, like localized time concentrations,

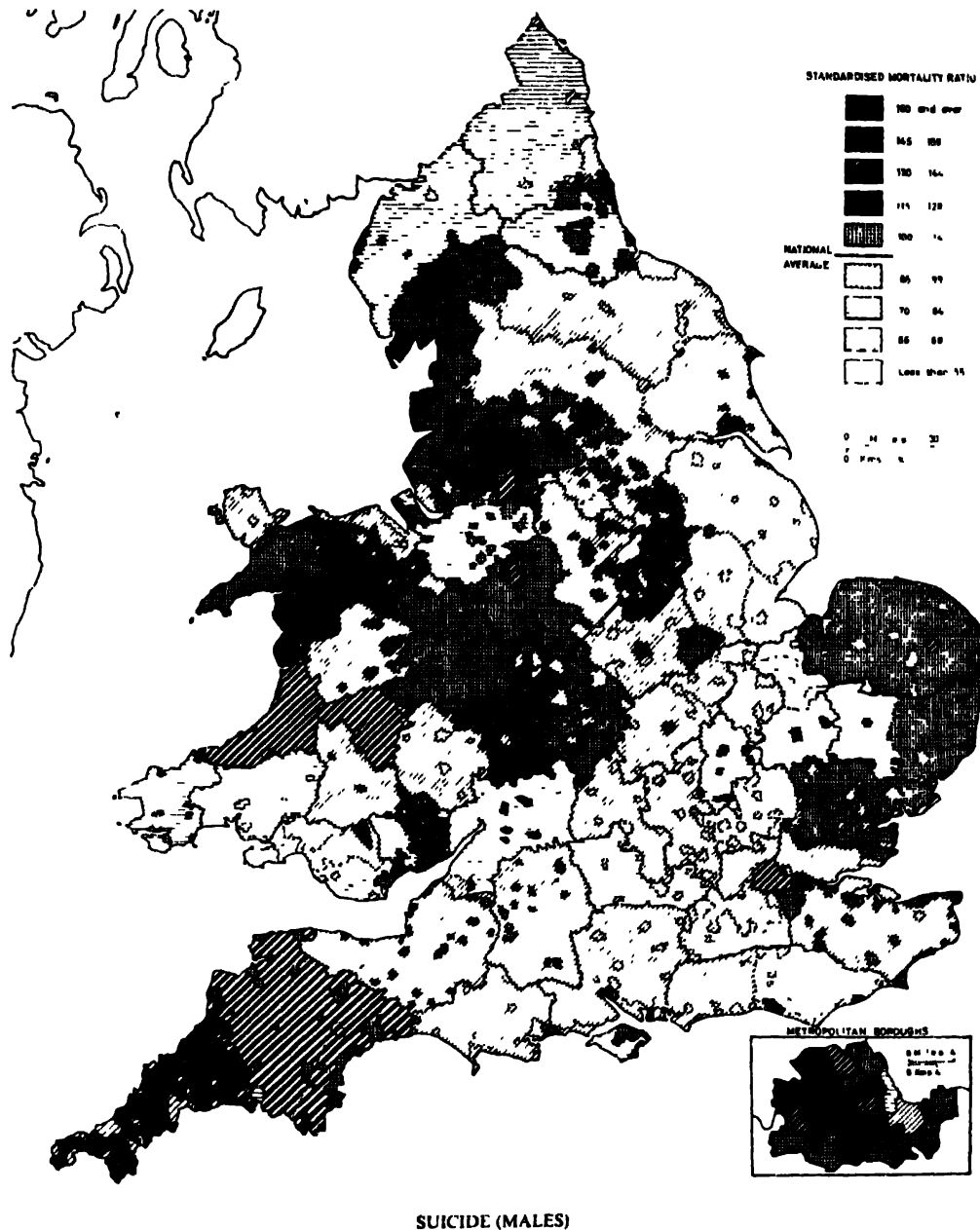


FIG. 12.2. Regional Variation in England and Wales of Age Standardized Mortality from Suicide. (From Howe, 1963.)

must almost always have environmental causes but with large scale geographical variations, such as carcinoma of the stomach and spina bifida, as with long term trends in time, it is always difficult to estimate to what extent the pattern may be a genetic one.

Parallel again with studies in time, the more interesting recent trends in geographical studies have concerned sparse events occurring at low intensity. To match the two cases of nephritis quoted above there is a recent account from the United States of two cases of rabies, four years apart, in tourists visiting some caves (Constantine, 1962). This observation led almost directly to the conclusion that the reservoir of the virus was in bats

and that biting was not a necessary stage in its transmission to man. As with low intensity time concentrations the geographical problem has two separate parts, the first, the detection of clusters of diseases (e.g. paratyphoid) known to behave in this way and the second, the detection of a propensity to occur in clusters in a disease of uncertain cause. This second problem is not one which has received much attention from a methodological point of view but if a concentration can be demonstrated in an area pre-defined in terms of some special risk then valuable interpretations can be made. This is analogous with attempts to link the occurrence of malformations with specific epidemics of infective illness. One recent excellent study (Newhouse & Thompson, 1965) demonstrates this principle on a geographical basis. An occupational investigation in 76 cases of pleural or peritoneal mesothelioma revealed 31 who had been exposed to asbestos dust in their work. Another 9 had suffered domestic exposure to asbestos dust from the clothes of relatives working in asbestos factories. Finally a group of 11 people, a much higher proportion than in controls, had no direct connection with asbestos processing but lived within half a mile of an asbestos factory.

### Space-Time Clusters

We have seen that there are formidable technical difficulties in demonstrating low intensity clustering in time and low intensity clustering in space in the absence of a well defined zone of prior risk. However, we cannot avoid the likelihood that there will be some diseases which exhibit low intensity clustering in time and space jointly. We can imagine an infective process smouldering and spreading from one locality to another and manifest only in a very small minority of the individuals involved in its transmission. In practice however, as with studies of time alone, the detection of the clusters seems to be easy; it is their validation which is difficult. Pleydell (1957) in Northamptonshire sparked off a controversy on this subject when he recorded intuitive impressions, unsupported by statistical argument, that certain malformations behaved in this way. Other authors (for example, Kellett, 1937; Wood, 1960; Heath & Hasterlik, 1963) have recorded similar impressions of leukæmia occurrences.

This problem contains a paradox in that the more data there are, the less easy is it to see, on an intuitive basis, that there is a tendency to spatial or temporal clustering at all. For example, we might find that in a single year in a particular town that six or seven cases of childhood leukæmia seem to be concentrated in two or three electoral wards. The next year we might find the same phenomenon except that they are different electoral wards and in a third year, different again. Plotting all three years on a single map, far from confirming the clusters by summation, diffuses the phenomenon so that it cannot be detected. Conversely, if the cases of leukæmia in a single ward or small group of wards are plotted in time we may find that there is a concentration in one particular year. We add a second geographical area, and find the same thing, but in a different year; as we extend the geographical bounds of our survey the temporal pattern vanishes. Consequently we find ourselves in the curious position of having to restrict the time of the study in order to demonstrate the geographical pattern and having to restrict the geographical area of the study in order to demonstrate a temporal pattern. With whooping

cough or measles we might have enough information to throw most of it away but with a rare disease we are in serious trouble.

The advent of computer techniques has transformed the situation by offering possibilities impracticable using traditional methods. A method suggested and applied by Knox (1964) is to examine every possible pair of cases, having first recorded the dates of occurrence and map references of the locations. For  $n$  cases there are  $n(n-1)/2$  possible pairs and each pair can be classified in two respects, first the time apart, and second the distance apart. A contingency table may be set up with arbitrary groupings of time and distance intervals and the whole set of data examined to see whether short distances are correlated with short times. If there is an interaction between the two it can be referred to appropriately as a space-time interaction and its presence implies a moving or creeping epidemiological pattern of the kind described. The concept of the space-time interaction is becoming widely accepted as a valid one but the most efficient and reliable statistical methods of detecting and confirming its presence are still matters for investigation. The present position is that the most simple and crude application, the construction of a  $2 \times 2$  table comparing short and long distances with short and long times, seems on several occasions to have detected an interaction in childhood leukæmia (see Table 12.II) (Knox, 1964; Spence Meighan & Knox, 1965). Applications to several other diseases, notably congenital malformations, have so far been negative.

TABLE 12.II

4560 PAIRS, FROM 96 CASES OF CHILDHOOD LEUKÆMIA  
IN NORTHUMBERLAND AND DURHAM

<i>Distances apart (km)</i>		<i>0-1</i>	<i>Over 1</i>	<i>Total</i>
Time apart (days)	0-59	5	147	152
	60-3651	20	4388	4408
Total		25	4355	4560

Expected  $< 1$  km and  $< 60$  days = 0.83  
Probability of 5 or more  $< 1/750$

It only remains to say that as with all other forms of epidemiological investigation, more than one biological interpretation can be put upon such a finding. Although we may speak of the phenomenon as demonstrating contagiousness in the statistical sense, we must not be misled into believing that this is synonymous with infectiousness. Space-time interactions occur in the distribution of poisons, irradiation, and diagnostic enthusiasms, as well as infections, and the general thesis developed throughout this discussion, that a biological interpretation is a non-formal process, applies here as elsewhere.

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## CHAPTER 13

# CARDIAC INFARCTION AND CARDIAC RESUSCITATION

by

P. G. F. NIXON

THERE has in recent years been a realization that many cardiac deaths can be avoided, if steps are taken promptly to deal with cardiac arrest, ventricular fibrillation and other dysrhythmias.

### CARDIAC ARREST

“Cardiac arrest” is the sudden failure of the ventricles to beat. In the past it was known that cardiac arrest might occur accidentally, but in general it was thought that the heart stopped beating when its useful life was finished. Occasionally direct cardiac massage restored cardiac activity, but it was rarely practiced and few patients survived. The idea that an electrical disorder might stop the healthy heart was not generally accepted until the advent of open cardiac surgery when it was realized that electrical stimuli can stop the heart, restore its beat, produce or remove ventricular fibrillation and relieve heart block. It was soon appreciated that anoxia can stop the heart, and, in cardiac arrest, oxygenation is required for the resumption of activity; that the injection of calcium into the circulation often strengthens weak beats; that ventricular fibrillation can more easily be converted to sinus rhythm when the size of the fibrillary movements has been increased by the use of adrenaline; and that metabolic acidosis inhibits the resumption of normal cardiac activity. Sometimes the heart is too weak to support the circulation after by-pass and death can be avoided if the stroke volume is increased by expansion of the blood volume, or the heart beat strengthened by adrenaline. A heart rhythm that is too slow and disturbed by much ectopic activity can often be restored to an acceptable rhythm with isoprenaline. It has also been appreciated that fear and pain, hypotension and anoxia predispose to cardiac arrest. Cardiac arrest is relatively uncommon in the absence of these predisposing factors or without the warning of abnormal rhythms and/or metabolic acidosis.

The lessons learned from study of the exposed heart can be applied to patients with ischaemic heart disease and other acute conditions encountered in medical and surgical practice.

The standard of the nursing care and observation received by patients after heart surgery has been seen to be needed in medical cases, and intensive care units have been created for the management of cardiac and respiratory cases, and for the care of unconscious patients.

A therapeutically optimistic attitude results from cardiac arrest being considered as a new disease, rather than as a mode of death.

**Pathological Physiology.** There are three varieties of the failure of the pumping action of the heart, namely, ventricular fibrillation, asystolic arrest, and heart-block. In ventricular fibrillation the ventricles do not pump, but "writhe" ineffectively. In asystolic arrest the beat of the ventricles ceases or becomes too feeble or slow to supply vital organs with an adequate amount of blood. In heart-block the ventricular rate may be reduced to a point where death will occur.

**Incidence.** The incidence of reversible cardiac arrest, as distinct from untreatable sudden death, is unknown. It cannot be determined until a large enough series of patients has been efficiently treated. At present it is reasonable to assume that 20 to 30 per cent of hospital deaths from first coronary attacks may be avoidable. In Charing Cross Hospital, a general hospital with 300 beds, the organized treatment of cardiac arrest allowed ten patients to be treated successfully and discharged home to their normal lives during the first year.

**Ætiology.** In the majority of cases of cardiac arrest it is not easy to incriminate any single major factor as the cause of the electrical disorder that stops the heart beat; usually a number of small factors appear to have summated and it is remarkable that these factors do not prevent the resumption of strong beating when adequate treatment is given.

Narrowing of a coronary artery, even in the absence of angina pectoris, myocardial ischæmia or acute cardiac infarction, is probably an important factor. Beck & Leighninger (1955) for example, have suggested that it may be difficult to produce ventricular fibrillation in an animal with normal coronary arteries by reducing the volume or the oxygen content of the coronary arterial flow. But when a branch of a coronary artery is constricted, these manoeuvres render the myocardium unstable, and a small stimulus may easily trigger off ventricular fibrillation.

In man also it is reasonable to assume that the conditions which reduce the coronary bloodflow cause ventricular fibrillation more easily when a branch of a coronary artery is narrowed. Most of the coronary blood flow takes place during diastole and depends largely upon the pressure head and the available time. The pressure head may be regarded as the difference of pressure between the aorta and the ventricular cavities, and the available time the duration of the diastole. Hypotension obviously reduces the pressure head but other factors, often unsuspected, also may reduce it. An ectopic beat, for example, or a period of nodal rhythm may raise the ventricular pressure in diastole and cause it to approach the level in the aorta (Fig 13.1). Ectopic beats and dysrhythmias may increase the frequency of partial or complete ventricular contractions and greatly reduce the diastolic filling time per minute.

Hypotension may result from pain and fear, from loss of effective blood volume, from drugs and perhaps from reflex action in certain types of cardiac infarction (Sleight, 1964; Grayson & Lapin, 1966). Clinical experience suggests that whereas a simple faint may be a trivial matter in youth, it may cause cardiac arrest in older people, presumably in those with narrowing of a coronary artery.

Acute hypotension leading to ventricular fibrillation may be responsible for the sudden deaths encountered in aortic valvular obstruction, cardiomyopathy, chronic ischæmic heart disease and hypertension: in these condi-

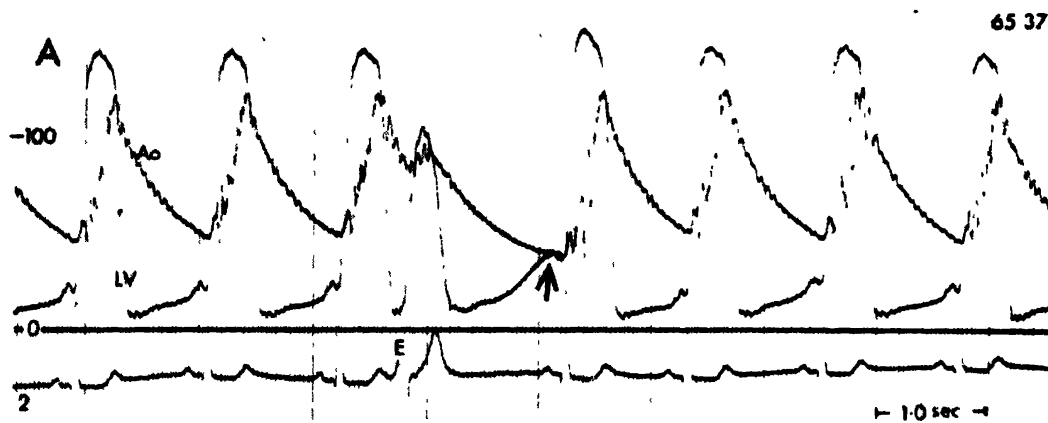


FIG. 13.1A.

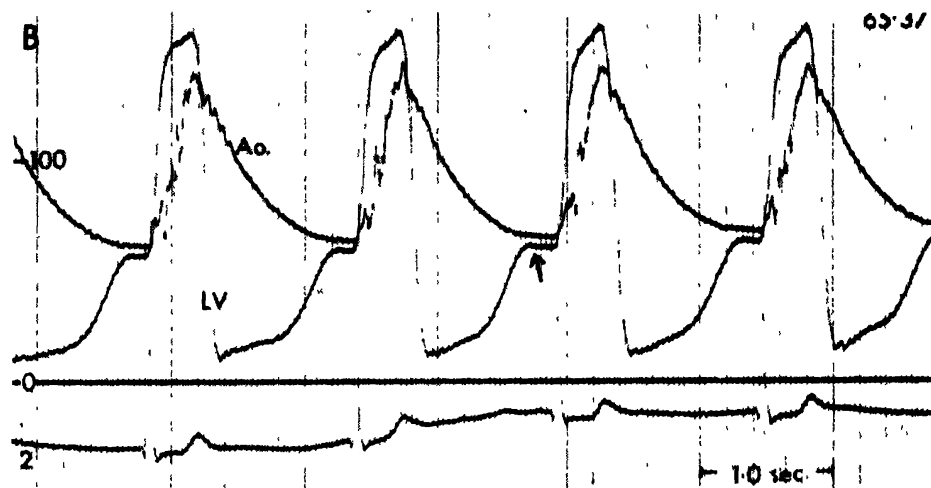


FIG. 13.1B.

FIG. 13.1. Simultaneous left ventricular and aortic pressure pulses and electrocardiogram from a patient with aortic valvar and ischaemic heart disease. In A the ventricular end-diastolic pressure (arrowed) rises to 40 mmHg after an ectopic beat; and in B, to 60 mmHg during a period of nodal rhythm.



FIG. 13.2. Pulmonary œdema in acute cardiac infarction.



FIG. 13.3. Acute cardiac infarction, showing the collection of œdema fluid in a patient nursed supine on a soft mattress, with his legs elevated.

tions study of the motion of the apex of the left ventricle suggests that the filling of this chamber depends upon a pathologically enlarged atrial contraction, and experience has shown that the loss of this powerful atrial contraction, as for example when a cardiac catheter initiates nodal rhythm, may be followed by a sudden and profound fall of the aortic pressure.

Hypoxia is a common factor in the genesis of cardiac arrest. Often it results from inadequate breathing after operation, or from pulmonary oedema in cardiac infarction. The oxygen undersaturation of the blood does not have to be severe to cause dysrhythmias and cardiac arrest, and cyanosis may not be obvious. Arterial oxygen estimations reveal the undersaturation, and these may also be a metabolic acidosis which appears to make the heart irritable and weak, and prone to dysrhythmias and arrest.

In cardiac infarction pulmonary oedema may result from a rise of the pulmonary venous pressure, which is the consequence of elevation of the left ventricular end-diastolic pressure. The rise in pressure responsible for hypoxia and metabolic acidosis need not be great enough to stiffen the pulmonary veins and cause dyspnoea at rest, nor to flood the lungs with fluid. The clinical signs in the lungs are often slight, and fail to suggest the presence of the widespread changes that may be seen radiographically (Fig. 13.2). In acute cardiac infarction with congestive heart failure it seems possible that hypoxia and metabolic acidosis may be caused by another variety of pulmonary oedema. Here the lung function appears to be disturbed by the fluid that has been led by gravity to accumulate in the chest when the patient is nursed on a soft mattress, either supine or with the legs elevated (Fig. 13.3).

The ectopic rhythms, which reduce coronary blood flow, may cause cardiac arrest in a different way. An ectopic beat may occur in the "vulnerable" period of the cardiac cycle, indicated by the T wave of the electrocardiogram, and cause the immediate onset of ventricular fibrillation (Julian, Valentine & Miller, 1964). Experience of cardiac catheterization suggests that pain and fear may predispose the patient to ectopic rhythms, particularly in the presence of ischaemic heart disease. Digitalis, it is now realized, is a frequent cause of dangerous dysrhythmias and cardiac arrest. The reason may be the widespread use of oral diuretics which deplete the heart of potassium and render it abnormally sensitive to the toxic effects of digitalis. Hyperventilation during intermittent positive-pressure ventilation may have a similar effect, and digitalis should be used with the greatest caution in patients undergoing artificial ventilation.

Hypoxia and metabolic acidosis appear to predispose the heart to dangerous dysrhythmias, and the administration of oxygen and sodium bicarbonate may restore sinus rhythm in patients with chaotic multifocal ectopic dysrhythmias and runs of ventricular tachycardia.

The autonomic nervous system can probably play a part in the genesis of cardiac arrest. Hypotension and bradycardia suggestive of parasympathetic overactivity, and tachycardia with a proneness to ventricular fibrillation suggestive of sympathetic overactivity, are uncommonly encountered at cardiac catheterization when the patient and the staff are tranquil. They are more common when the patient is tense and fearful, and subjected to sudden disturbance from pain or noise.

Most sedative and analgesic drugs reduce the peripheral vascular

resistance, and can cause hypotension, syncope and cardiac arrest if the head-end of the patient's bed is raised.

Quinidine may cause ventricular fibrillation without any warning from symptoms, signs, or electrocardiographic changes, even when the dose is as small as 300 mg.

**Symptoms.** Cardiac arrest may cause abrupt loss of consciousness, or it may follow a period of bradycardia, hypotension, pallor and fear.

**Physical Signs.** A lifeless appearance, absence of the carotid pulse and dilatation of the pupils are the most important signs. Respiratory movements may continue after the heart has stopped.

**Complications.** Death soon follows cardiac arrest in the untreated case. From the moment of the arrest the brain and the heart begin to suffer from the lack of circulation, and the chance of recovery becomes smaller with every minute that passes. With delayed and inadequate treatment the result may be an irreversible condition of low cardiac output or permanent brain injury.

**Diagnosis.** The diagnosis is made from the physical signs, and from the appropriate special investigation, the electrocardiogram.

**Prognosis.** The health of the patient appears to be unaffected by the incident of an adequately treated arrest, and there is no reason for believing that permanent harm follows the speedy treatment of the temporary electrical disorder. Where the heart has been free from intrinsic disease at the time of arrest, no abnormality has been detectable after recovery. When the arrest has occurred in the course of cardiac infarction, the patient may afterwards be capable of returning to normal activity.

### ( Emergency Treatment of Cardiac Arrest

When a patient unexpectedly collapses lifeless, the bystander instinctively potters about. He sends for help, spends too long attempting, for example, to take the blood pressure, mistakes the agonal sighing for a sign of life or calls for coramine. The first person to see the arrest must act in a determined manner within a matter of seconds. He must be confident in his knowledge of the treatment, and in the good order of his equipment.

The instruction sheet reproduced in Appendix 1 is employed in Charing Cross Hospital. It divides the treatment into a number of phases.

**Phase I:** In this phase the patient is made ready for resuscitation. Clearing the airway may save the patient who has been choked by a bolus of food, or by the tongue falling back. Raising the legs may save the patient whose arrest began as a faint from pooling of blood in dependent limbs (Woodward, 1960). A strong thump on the sternum may often provide sufficient mechanical stimulation for the restoration of the heart beat in the early moments of asystolic arrest (Scherf & Bornemann, 1960).

**Phase II:** Pressing down the sternum compresses the heart against the spine and ejects its contents, and the release of pressure allows the heart to fill. The "first-aider" must define the centre or the lower part of the sternum (not the xyphoid process), place on it the heel of one hand covered by the heel of the other, with the fingers elevated as in Fig. 13.4, and intermittently press downwards. The rate may be 80 to 100 strokes per minute. The force must be sufficient to produce a carotid pulse and keep the pupils small; in

adults the sternum is pressed down a distance of 3 or 4 cm (Kouwenhoven, Jude & Knickerbocker, 1960). The viscera escape injury when the pressure is applied directly downwards over the centre or lower part of the sternum, and the force is the least amount that will produce a carotid pulse and prevent pupillary dilatation. Fractures of the costal cartilages may occur in the elderly, but tend to heal rapidly under treatment with intermittent positive pressure ventilation.

In order to perform mouth-to-mouth artificial respiration, the patient's chin is elevated and his neck extended to prevent the tongue from falling back and obstructing the airway; the patient's nostrils are closed and the resuscitator takes in a breath, applies his mouth to the patient's over a handkerchief, and exhales to fill the patient's lungs. It is the usual practice to inflate the lungs once after every ten sternal compressions. Contact with the patient's lips may be avoided by the use of a Brook Airway (Brook, Brook & Wyant, 1962), (Fig. 13.5), or similar device. Mouth-to-mouth insufflation may be obviated by the use of a bag or bellows fitted with a facepiece.

The signs of efficient artificial respiration and cardiac massage are smallness of the pupils and pinkness of the mucosæ, perhaps with returning consciousness. When these signs have been produced the immediate urgency has passed, and the manoeuvres of phase III can be performed without indecent haste.

Spontaneous reversal of ventricular fibrillation, with complete recovery of the patient, has occurred after 50 minutes of this first-aid treatment (Wetherill & Nixon, 1962).

All hospital staff, however junior, should be trained in this procedure and instructed to continue it without breaking off to seek help.

**Phase III:** The trachea is intubated for more efficient artificial respiration, an intravenous infusion is set up for the administration of drugs, and the nature of the electrical disorder is diagnosed electrocardiographically.

**Phase IV:** Sodium bicarbonate is given intravenously to correct the metabolic acidosis that predisposed to the arrest or occurred as a result of it. Analysis of the arterial blood may show that as much as 300 ml of an 8.4 per cent solution are required. Calcium is given empirically for there appears to be benefit from it and never harm.

Every compression during closed cardiac massage causes a deflection of the stylus of the electrocardiograph. In complete asystolic arrest no other deflections occur, the Q.R.S. waves being absent. In other cases infrequent and grossly abnormal Q.R.S. complexes may be seen when the massage is interrupted for a moment, but the ventricular contractions which take place do not give rise to palpable pulse. In some cases of arrest the heart beat may never recover, but in others it will. It is difficult to decide when treatment should be abandoned; complete recovery has been observed 20 minutes after an asystolic arrest (Nixon, 1961). When the heart does not recover from asystolic arrest with treatment it is usually found at autopsy that the myocardium has been severely damaged by previous infarction. In resistant asystolic arrest internal and external pacemaking and internal cardiac massage have not been useful. Holmdahl (1965) has had some success with injecting large quantities of adrenaline to produce ventricular fibrillation which is amenable to electrical conversion into sinus rhythm.



Ventricular fibrillation (Fig. 13.6) presents as an irregular wave form in the electrocardiogram. If the waves are rapid and small, adrenaline will cause them to slow and enlarge, and then electrical defibrillation is more easily accomplished. Most workers prefer a direct current discharge to an alternating current shock.

Every defibrillating instrument should have an instruction sheet to describe its method of application, but there are several principles which should be observed, whatever the variety of the machine. To avoid burning the chest wall it is necessary to apply electrode jelly and ensure that the whole area of each electrode is pressed firmly against the skin.

Smaller shocks are more effective when one electrode is placed between the scapulæ and one over the centre of the sternum than when both electrodes are placed on the anterior chest wall. The first shock to be applied should be at the lower level of the range indicated for external use, but if this is not successful, further shocks of increasing power should be given until the fibrillation is reversed. It is essential to oxygenate the myocardium as well as possible with vigorous massage and ventilation until the moment before the shock is given. Care must be taken to exclude all bystanders from conduction of the shock. The electrodes begin to charge again as soon as the shock has been applied, and they should be regarded as *ALIVE* and *DANGEROUS* until they are disconnected from the machine.

The first beats after a period of arrest may be brisk and strong, but often they are weak, and they may fade out unless the myocardial oxygenation is maintained by the continuation of the massage and lung inflation. The artificial circulation must be maintained until the heart's own beat is strong enough to produce an easily palpable carotid pulse, and to keep the pupils small. The continuation of the massage to this point does not prejudice the heart-beat, and premature cessation of the massage may be followed by a return of the fibrillation.

In heart surgery, when ventricular fibrillation is deliberately produced and reversed, hypoxia and metabolic acidosis increase resistance to defibrillation. Hence, in the resistant case of cardiac arrest, it may be more profitable to attempt better oxygenation and correction of the acidosis than to persist in giving large numbers of powerful shocks. Opening the chest and placing the electrodes on the heart has no advantage over their external application.

Propranolol 2 mg intravenously, may prevent repeated relapses into ventricular fibrillation. Sloman, Robinson & McLean (1965) used the drug for the relief of recurrent and resistant ventricular fibrillation in acute cardiac infarction, although in a dosage that may have been too large, and Seaton (1966) for a similar complication of quinidine therapy.

**Phase V:** In most surviving cases treatment began with the minimum of delay, the pupils never dilated, the heart beat was brisk from the first moment of its restoration, breathing began spontaneously and continued adequately, the blood pressure climbed to a satisfactory level within a few minutes, and consciousness speedily returned. In a minority the blood pressure remained low for a period of hours, and required elevation with a pressor drug or infusion. The chief danger from hypotension is the defective filling of the coronary arteries which weakens the myocardium, and tends to cause a repetition of the arrest.



FIG. 13.4. Closed-chest cardiac massage. The position of the hands.



FIG. 13.5. Mouth-to-mouth artificial ventilation with a Brook airway. Note the closure of the nostrils and the elevation of the chin.

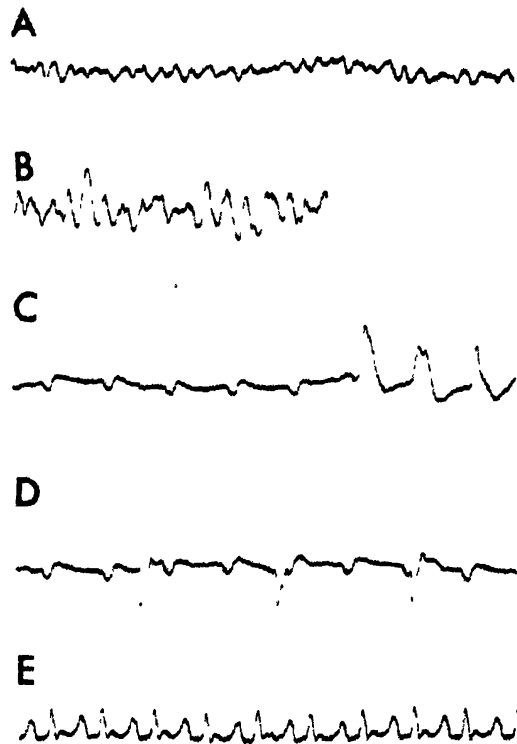


FIG. 13.6. The treatment of ventricular fibrillation. ECG strip (A) shows small-magnitude fibrillation, enlarging after adrenaline (B). (C) is recorded immediately after the DC shock, and (D) and (E) two minutes and five minutes afterwards.

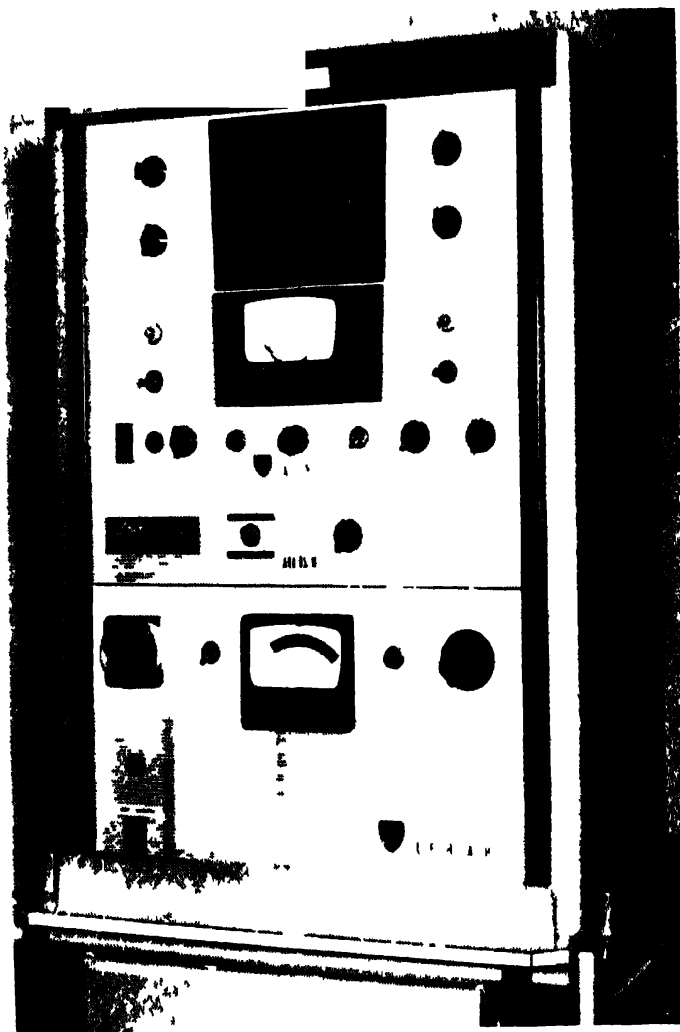


FIG. 13.7. The Lown apparatus



FIG. 13.8. The M.C. mask.

If the patient is weak and hypotensive, the peripheral circulation poor, the breathing inadequate, or if fracture of costal cartilages has produced a "flail" sternum, it may be desirable to rest the patient as much as possible for a day or two by taking over the work of breathing with a mechanical ventilator. In order to do this, the level of consciousness and the cough reflex are depressed with drugs and a cuffed endotracheal tube is introduced through the mouth and larynx and connected to a ventilator. The endotracheal tube can be left in place for as long as 48 hours. Tracheostomy should be restricted to cases where it is obvious that artificial ventilation will be required for more than two days. During artificial ventilation through an endotracheal tube, drugs are used to abolish discomfort and struggling against the machine rather than hyperventilation, for hyperventilation sometimes appears to increase the sensitivity of the heart to digitalis, and thereby increase the risk of dangerous dysrhythmias. The dosage of the drugs is probably more important than their choice. They should be given intravenously, and frequently enough to maintain an adequate effect, i.e. to allow the patient's breathing to be submitted to the machine. Omnopon may be used in alternation with promethazine hydrochloride and pethidine; phenoperidine and droperidol have recently proved satisfactory.

With adequate ventilation and drug therapy the patient should appear to be sleeping contentedly, and the warmth and pinkness of the fingers should indicate the adequacy of the oxygenation, the cardiac output, the blood volume and the peripheral circulation. The absence of a "negative phase" on the ventilator may hinder the return of venous blood to the heart, and cause the venous pressure to rise and the arterial pressure to fall. These signs sometimes lead to the erroneous diagnosis of cardiac tamponade.

The trachea and main bronchi should be sucked out with sterile catheters, using a no-touch technique, as and when it is necessary. Some workers deflate the cuff of the endotracheal tube for a minute or two every hour to interrupt its compression of the tracheal mucosa.

Ideally a ventilator should be volume-cycled rather than pressure-cycled, and a simple pressure gauge should permit the pressure level created by the delivery of each given volume to be read off at a glance. Increases of the pressure usually indicate an airway obstruction or acute pulmonary oedema. The machine should be capable of exerting a pressure of up to 70 cm water because patients with acute pulmonary oedema may have pulmonary venous pressures of 40–50 cm of water. The ventilator ought to have a simple control to set the stroke volume and another to select the number of respirations per minute. Ventilators that meet these requirements, and do not require too high a degree of nursing skill for supervision, are the Air-shield and the Cape. In practice the stroke volume and the respiratory rate that will be required by the patient are initially guessed. After some minutes of ventilation the blood oxygen saturation and  $\text{PCO}_2$  are measured. With the knowledge of these values the machine can be adjusted accurately to the needs of the patient.

When delayed or inefficient treatment has occurred the patient may be comatose, and the plantar reflexes extensor. The cerebral changes that cause this condition are amenable to treatment. If the breathing is inadequate a ventilator is employed in such a way as to guarantee the maximal possible oxygenation while avoiding the cerebral vasoconstricting effect of hyper-

ventilation. The head of the bed is elevated, a few degrees at a time, to the highest level that is practicable (too rapid or great an elevation may cause the blood pressure to fall and the fingers to lose warmth and pinkness). Elevation of the bed-head may have a dramatic affect, the patient regaining consciousness within minutes of it being raised to about 45°, only to relapse when the bed is lowered again. The bladder is catheterized, and urea (80 g in 200 ml five per cent glucose solution) is given intravenously to "dry" the patient. The respiratory needs of the patient are reduced by cooling; the patient is covered with a wet sheet, and exposed to a vigorous draught from two or three fans. The rectal temperature is reduced to 32 to 33°C for two or three days, and the patient is allowed to warm when he regains consciousness. Promazine hydrochloride 10–20 mg intravenously is given when necessary to prevent shivering.

In a series of ten successfully treated cases of cardiac arrest five required the use of a ventilator, and in three the delayed return of consciousness made cooling obligatory.

There is unusual susceptibility to infection after cardiac arrest, particularly to pneumonia if pulmonary œdema is present, and it is reasonable to give a wide-spectrum antibiotic for five to seven days. There is a risk of deep venous thrombosis, therefore the calves should be massaged and the legs moved passively every four hours until active breathing and leg exercises can be carried out.

#### **Communication and the Emergency Treatment of Cardiac Arrest**

The creation of intensive care units is likely to reduce the incidence of cardiac arrest in hospital because warning signs will be observed and appropriate treatment given before the heart stops beating. In the general wards the warning signs are often not recognized and the survival of the patient depends upon the speed with which treatment can be applied after the heart has stopped beating. Clearly both a system of calling a specialist team rapidly and the ready availability of the resuscitation apparatus will improve the results.

The arrest may have to be treated at the place of its occurrence, but it is preferable, once the trachea has been intubated, to move the patient by trolley to a separate room. Closed-chest massage can be continued by an operator who kneels astride the patient and moves with the trolley.

#### **Equipment Required for the Emergency Treatment of Cardiac Arrest**

The emergency equipment listed in Appendix A should be kept in a box in every hospital ward.

Other equipment for emergency intubation of the trachea, listed in Appendix B, may be kept in boxes situated at the junctions of main corridors and in special departments.

The boxes are closed with easily broken fuse-wire seals. The seals should be inspected every day and the contents of the boxes with broken seals checked and replenished.

When a cardiac arrest call is put out an anæsthetic trolley and resuscitation apparatus are brought to the patient. Many varieties of resuscitation apparatus are available. The simplicity of operation is the most important factor and it

is unfortunate fact that the easiest models to use may be the most expensive. The apparatus pictured in Fig. 13.7 is very satisfactory. It is mains-driven, and connected to the patient by means of skin-electrodes. The electrocardiogram is displayed on a cathode-ray tube, and the R or S waves may be set to trigger a flashing light, to give a noise signal, or to operate a meter indicating the heart rate. The apparatus can be set to give audible warning of a deviation of the heart rate outside selected upper and lower limits. The pace-making portion of the apparatus can be used externally or internally, and it can be set to operate automatically when the heart rate falls below a chosen level. The DC defibrillator component can be used to correct ventricular fibrillation. The condenser discharge can be synchronized, and used to convert a dysrhythmia to sinus rhythm.

Other equipment that may be required in the investigation or treatment of cardiac arrest will be discussed under "Intensive Care and Investigation" (see page 409).

### **Prevention of Cardiac Arrest with Particular Reference to the Management of Acute Coronary Disease**

The importance of fear, pain and discomfort in provoking abnormal electrical cardiac states has been emphasized. A great deal can be done even in general wards to minimize these. Reassurance, peace and quiet are invaluable to the patient with acute coronary disease, who should be nursed in a comfortable position, and allowed oral fluids and a light diet as he desires. He should be encouraged to move about in bed as he wishes, and be trained in the breathing and leg exercises that reduce the incidence of deep venous thrombosis and pulmonary embolism. Oxygen may be given by means of an MC mask (Fig. 13.8), and its use is strongly to be desired in the more severe cases.

Retention of urine may be prevented by allowing the elderly patient to put his legs over the side of the bed when he uses a urine bottle and the bedside commode is preferable to the bedpan.

In acute cardiac infarction it is desirable to keep the patient under heavy sedation for the first two or three days. An intravenous infusion of five per cent glucose solution, at a minimal flow-rate, may be used without disturbing the patient, not only for the administration of sedatives and other drugs, but for giving heparin and for the measurement of the venous pressure with a saline manometer. Promethazine hydrochloride and pethidine, 25 to 50 mg of each, may be given intravenously often enough to keep the patient in a drowsy and tranquil state without causing hypotension. Alternatively, droperidol 5 to 10 mg daily, and phenoperidine 1 mg given as often as necessary, are now under trial and appear to be satisfactory.

The most important part of the clinical examination in acute cardiac infarction is palpation of the apex of the left ventricle when the patient is lying on his left side. In almost all cases the systolic push of the ventricle is preceded by a presystolic tap. The less frequently audible atrial or fourth heart sound consists of the audible vibrations set up by this tap, which is caused by an abnormally powerful contraction of the left atrium. This is the earliest recognizable sign of left ventricular "failure" or "distress", and is an indication for careful digitalization. The patient may be allowed out of bed



when this sign disappears, or reduces to a minimum, but should return for more rest if it reappears.

Mason Sones (1966) recommends the administration of isosorbide dinitrate (Vascardin) every three or four hours to promote the coronary blood flow. Sones has confirmed Gensini's (1963) observations of the potency of this long-acting coronary vasodilator.

Baroldi (1965) examined the hearts of 449 cases of sudden coronary death, or of death with acute or recent myocardial necrosis, and considered that thrombosis caused neither the necrosis nor the sudden death. In the minority of cases in which it occurred it was more likely to have resulted from the particular hæmodynamic conditions existing in and around a diseased artery. Baroldi's findings question the basis of the use of anticoagulants in acute coronary disease, and Wasserman and co-workers (1966) are not alone in finding that their use does not reduce mortality. However, heparin may be given intravenously during the first two or three days after admission, while the patient is obviously ill, and followed by physiotherapy and early mobilization to prevent phlebothrombosis and pulmonary embolism.

The causes of hypoxia and hypotension should be sought and corrected wherever possible. In cardiac disease pulmonary œdema is the commonest cause of hypoxia. The patient is usually frightened and reassurance is important; sedation, diuretics and digitalis may be used. Oxygen should be given and the upper part of the body should be raised to drain œdema fluid from the chest by gravity if the patient is free from signs of deficient cerebral blood flow. Severe interstitial œdema of the lungs may cause surprisingly few physical signs, and a portable radiograph of the chest should be taken if hypoxia is suspected (Figs. 13.2, 13.3). Ectopic rhythms and the loss of effective atrial contraction in atrial fibrillation may contribute greatly to the pulmonary œdema by raising the left ventricular diastolic pressure, and they should be corrected as soon as possible.

In pulmonary œdema with turgidity of the pulmonary veins and distressing and exhausting dyspnœa, it is often desirable to induce sleep, intubate the trachea, and use artificial ventilation with a volume-cycled intermittent positive-pressure ventilator. This treatment rests the patient as much as possible, and allows adequate ventilation and oxygenation.

Hypotension is a potent cause of cardiac arrest, probably because it reduces coronary blood flow and tissue perfusion, and promotes metabolic acidosis. It is not rare for the blood pressure to fall after the patient is admitted to hospital, but it is not necessary to take any action if the hands and feet remain pink and warm, if the cerebral circulation is adequate, if the urine flow is maintained and if the patient's general appearance is satisfactory. The systolic blood pressure may fall to 70 or 80 mm Hg for a few hours, and then rise again of its own accord without harm.

In some cases hypotension is associated with peripheral vasoconstriction, oliguria, mental disturbance and metabolic acidosis. In these patients oxygen must be given and steps taken to determine the cause of the hypotension. There are many causes of this in acute coronary disease, ranging from fear to tamponade. Once identified the cause should be treated; the haphazard use of nor-adrenaline should be avoided.

Hypotension may result from a syncopal reaction, particularly when

sedatives and analgesics have been given, and a satisfactory blood pressure and circulation may be restored by raising the legs.

Hypotension may be associated with bradycardia from slow sinus or nodal rhythm; this precursor of asystolic arrest may often be abolished by the intravenous injection of 0.6 mg atropine sulphate. The restoration of the heart rate with atropine is not always accompanied by the recovery of an adequate blood pressure and circulation. Isoprenaline may be effective in these circumstances; 2 to 3 mg of isoprenaline in 100 ml five per cent glucose solution are given by intravenous drip at the slowest rate that suffices to correct the bradycardia and hypotension. Treatment is continued until the patient can be weaned off the drug; this may be up to two or three days. Extreme care is needed to avoid ventricular fibrillation which may result from overdosage.

Cardiac arrest is a common occurrence in the hypotensive cases of posterior cardiac infarction with heart block. The circulatory failure may respond to treatment with isoprenaline, but the author prefers to insert a pacemaking catheter into the heart, and electrically to maintain an adequate heart rate. If restoration of an adequate heart rate does not improve the blood pressure and the circulation it may be necessary to use an inotropic agent to augment the heart beat, or in certain carefully-controlled situations, to expand the circulating blood volume. The inotropic agent adrenaline is used in a dilute solution, 3 ml of 1/1,000 solution being added to 500 ml of five per cent dextrose in water, and given intravenously at the slowest effective rate. The patient is weaned off the drip as soon as possible, and the overdosage that might cause ventricular fibrillation is carefully avoided by close attention to the drip-rate.

The vasopressor drugs and nor-adrenaline may have a place in the treatment of some of the hypotensive states of acute coronary disease, but their role is not established. Patients with hypotension from acute heart disease generally have intense peripheral vasoconstriction; blood volume studies do not suggest the internal pooling of blood, and the use of vasopressor drugs and nor-adrenaline is not rewarding (Editorial, *Brit. med. J.*, 1966). The chief therapeutic difficulty is the empiricism of our methods, which generally consist of attempts to apply to coronary disease the lessons that have been learned in heart surgery. The methods necessarily are empirical because the left atrial pressure, the cardiac output and the peripheral resistance cannot be measured continuously, or with ease and safety in sick patients; and relatively little is known about the mode of action of the powerful agents that may be applied to the various syndromes of severe coronary disease.

### CARDIOGENIC SHOCK

The term shock in acute myocardial infarction is used here to define a syndrome of pallor, hypotension, restlessness and disturbance of consciousness, intense peripheral constriction and anuria, with or without elevation of the central venous pressure. Metabolic acidosis invariably occurs. Death is likely to follow these signs, unless they arise from a syncopal reaction that can be treated by altering the posture, or from a dysrhythmia that can be corrected. The incidence of shock in cardiac infarction is about

15 per cent, and the mortality is said to be about 88 per cent (Epstein & Relman, 1949). The true mortality is probably nearer to 100 per cent because authors may have included cases of syncope, or of hypotension secondary to a dysrhythmia.

It is worthwhile to treat the hypotension because it predisposes to metabolic acidosis, dangerous dysrhythmia and renal failure, reduces the collateral blood flow to the ischaemic area (Estes *et al.*, 1966), reduces the contractility of the myocardium supplied by narrowed coronary vessels (Hellerstein, Brofman & Caskey, 1952) and increases ballooning of the infarcted area in systole (Corday, Bergman & Kruger, 1949). The syndrome is fortunately commoner in branch occlusions of a coronary artery than in main-stem obstruction (Kurland, Weingarten & Pitt, 1965), and surprisingly rapid and complete recovery occurs in successfully treated patients (Nixon, Ikram & Morton, 1966 and 1967).

Treatment has consisted variously of transfusion, vasoconstrictive drugs and inotropic agents. Transfusion passed out of favour when blood volume measurements failed to show a deficit, but vasoconstrictive drugs were said to be beneficial even though intense vasoconstriction is the most striking sign. Vasoconstrictive drugs do not save advanced cases nor do they halt the progression of the metabolic acidosis that indicates the failure of the circulation.

Vasoconstrictive drugs with an inotropic action have yet to prove their value in cardiogenic shock. Their greatest effect may be in patients who are syncopal from the sedative drugs that encourage the pooling of blood in the patient nursed head up. It is often difficult to wean patients off these drugs, and infusion is required before their administration can be stopped (Botticelli, Tsagaris & Lange, 1965). Experience suggests that infusion alone may be adequate treatment.

The author's cases with advanced signs and raised central venous pressure (CVP) were nursed supine and given oxygen to breathe. Five per cent glucose solution was given intravenously by drip in doses of 50 to 200 ml at a time. The rate of infusion was controlled by observation of the CVP, the arterial pressure, the urine flow, and the clinical appearance of the peripheral circulation. It was not difficult to resuscitate each patient by using the lowest rate of infusion that improved the arterial pressure and the peripheral circulation, and restored the urine flow. Pulmonary oedema created neither a clinical nor a therapeutic problem, although transitory early signs may have appeared on radiographs.

Each dose of the solution raised the arterial pressure, and also the venous pressure. The latter was allowed to subside towards its original level before the next dose was given. The technique is reminiscent of the treatment of hypotension at the end of heart and lung by-pass operations where blood is injected from the machine until a satisfactory level of arterial pressure is achieved. The venous pressure obtained at this point is maintained by intravenous infusion. If the injection from the machine fails to improve the arterial pressure, the venous pressure rises "uselessly" and the procedure is abandoned.

After the resuscitation it was necessary to continue the infusion for varying periods before the tendency to hypotension disappeared and left a stable

circulation. Typical cases have required a total of 4.1 l solution in 20 hours, 2.1 l in 12 hours, 12.3 l in one week.

The following case exemplifies the method:

A 76-year-old man collapsed suddenly and was brought to hospital two hours later at 7 pm. He was extremely ill and pale; the circulation in the ears, fingers and toes was greatly reduced; the systolic blood pressure lay between 90 and 100 mm Hg; the mental condition varied between stupor and semi-conscious restlessness, and the rapidity of his deterioration was obvious. He had been at work earlier in the day and was preparing for his marriage. During his transfer to an intensive care suite the heart rate slowed to 40 per minute, the trunk became mottled with cyanotic patches of stasis, and the fingers and ears blue. His supine position was maintained and the legs were elevated; oxygen was given by face-mask, and the atropine sulphate 0.5 mgm

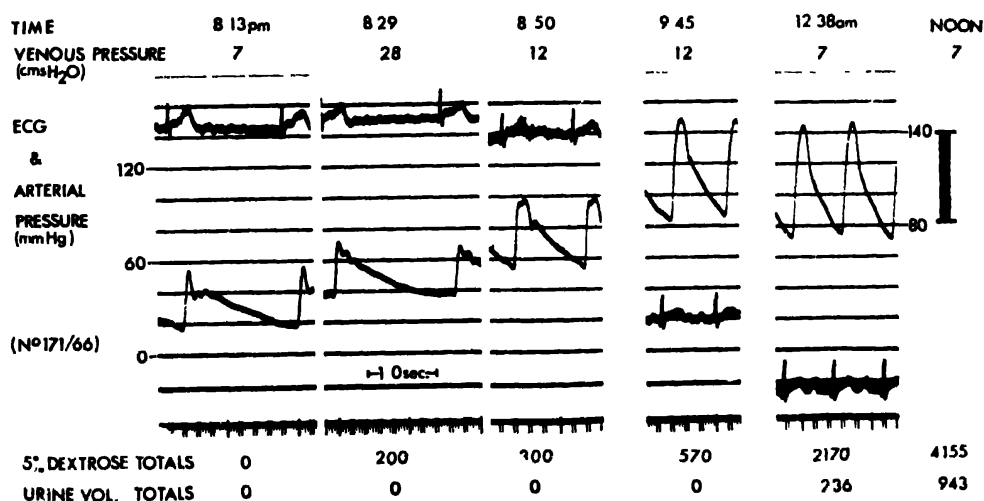


FIG. 13.9. Records of the central venous and arterial pressure, the ECG, the urine flow and the dextrose infusion from a patient treated for cardiogenic shock.

was injected intravenously. His deterioration continued. A cardiac-catheter was introduced into the superior vena cava from an arm vein, an indwelling needle was inserted into the brachial artery and the bladder was catheterized.

Arterial blood analysis showed pH 7.35; oxygen saturation 97 per cent; and a metabolic acidosis of minus 6.5 mEq/l as determined by the Siggard-Anderson nomogram. The acidosis was corrected with an intravenous infusion of 8.4 per cent sodium bicarbonate solution. The arterial pressure recorded with an electromanometer was 55/18 mm Hg, and the CVP 7 cm water above the sternal angle (8/13 pm, Fig. 13.9). No urine was formed. The electrocardiogram showed slow atrial fibrillation with ectopic beats, and a marked current of injury.

At 8.13 pm an infusion of five per cent glucose solution was begun into the superior vena cava, and the first 200 ml was given in ten minutes. This raised the CVP to 28 cm water, but also raised the arterial pressure to 70/40 (8.29 pm, Fig. 13.9). In 16 minutes the CVP fell to 12 cm water, and further doses of 100–200 ml five per cent glucose were given. The arterial pressure reached a peak of 150/80 (9.45 pm, Fig. 13.9), the heart rate increased

and the change in the shape of the arterial pressure pulse was consistent with a marked increase in the stroke-volume.

Each successive dose of the glucose solution raised the venous pressure by a smaller amount and for a shorter period until, at 01.14 am, five hours after the treatment began, 200 ml. were absorbed in two minutes without affecting the CVP. The arterial pressure rose from 80/44 to 110/60 (Fig. 13.10).

The raising of the arterial pressure with the treatment was accompanied by other signs of improvement. The first was recovery of mental clarity and calm after half-an-hour of transfusion. After two hours the urine flowed, and the ECG current of injury diminished. After four hours the peripheral circulation seemed normal, and the atrial fibrillation reverted to sinus rhythm (12.38 am, Fig 13.9).

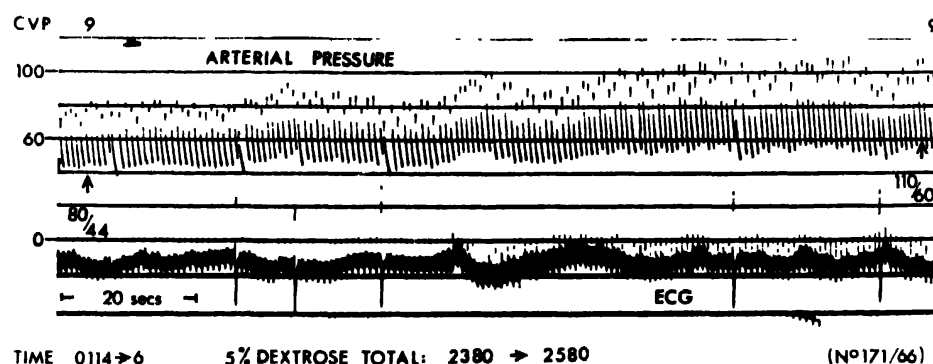


FIG. 13.10. The central venous and arterial pressure and the electrocardiogram during the infusion of 200 ml 5 per cent dextrose during a period of 2 minutes.

By noon on the day after admission, he looked well, and took an alert interest in his surroundings. The blood pressure was stable at 140/80 without transfusion and the CVP was 7 cm water. The transfusion was stopped, a total of 4,155 ml having been given in 16 hours, during which 943 ml urine were passed.

Four days later he was active in bed with a blood pressure of 160/85; and the CVP 2 cm water. He has since returned to normal activity and is free from heart failure.

It may be considered that this patient, and others (Nixon, Ikram & Morton, 1966 and 1967) recovered spontaneously despite the massive infusion, but it appeared that he was dying and that the infusion resuscitated him.

Another patient was similarly resuscitated but died suddenly during convalescence. Two others failed to respond to the infusion, and neither nor-adrenalin nor adrenaline had any clinically detectable effect upon the circulation. Autopsy revealed severe myocardial damage from previous infarctions.

It has been suggested that the presence of congestive heart failure in cardiogenic shock indicates a "limited reserve of the circulatory system", and that intravenous infusion may be injudicious and dangerous, "over-dilating" the heart, further reducing the cardiac output and aggravating the shock (Berman & Akman, 1952). In the patient described here the central

venous pressure was abnormally high (7 cm H<sub>2</sub>O) at the start of the treatment, and yet the restoration of the blood pressure, peripheral circulation and urine flow, and his recovery, suggest that the transfusion was beneficial. This raises the question as to whether the elevation of the venous pressure really is a sign of congestive heart failure or whether it indicated some other phenomenon. Most probably it is a sign of the constriction of the venous system, the evidence being the great increase in the venous pressure, from 7 to 28 cm H<sub>2</sub>O, when 200 ml five per cent glucose first were added to the circulating blood volume. This contrasts with the response at a later stage, after the circulation had improved with the infusion of 2,380 ml five per cent glucose, when a dose of 200 ml was absorbed without altering the central venous pressure, presumably because the venous constriction had disappeared.

The explanation of the beneficial effect of infusion may lie in the experimental observation of Ross (1966). He has confirmed the "ballooning" of the wall of the recently infarcted ventricle, and shown that such a chamber requires distension before its output can be restored to maintain the pre-infarct level of arterial pressure. Perhaps the infusion resuscitated the case referred to above by distending the heart to the point at which its output was adequate for the maintenance of life. Clinically there are many similarities between hæmorrhagic and cardiogenic shock. In the former, the important disturbance is a deficiency of the effective blood volume; in the latter the important disturbance may be a sudden relative deficiency of the blood volume in relation to the needs of the newly-injured heart.

Ectopic activity is sometimes abolished or minimized under treatment with infusion. One reason for this may be an improvement in the coronary circulation consequent upon the blood volume expansion. Another reason may be the potassium-shifting or biochemical effect of the infused glucose (Sodi-Pallares, 1961; Mittra, 1965).

Infusion treatment is only indicated in a desperate clinical situation where death seems imminent, and where the alternative methods have already proved valueless. Infusion may be extremely hazardous in acute infarction, and it should be attempted only where there is an adequate standard of intensive care, and where the complication of pulmonary œdema, if it occurs, can be treated with intermittent positive-pressure ventilation.

Some of the patients who appear to be dying from cardiogenic shock recover with infusion, while others die with irreparably damaged hearts. It is not possible to distinguish these, but it is suggested that a solution of 3 ml of 1/1,000 adrenaline hydrochloride in a 500 ml unit of five per cent glucose may be dripped slowly intravenously in cases where one or two doses of glucose solution have proved ineffective. The shocked case that responds neither to the volume-expanding and biochemical effects of the glucose nor to the powerful inotropic effect of the adrenaline probably has too little viable myocardium to survive without the help of an artificial circulation.

### ECTOPIC RHYTHMS ✓

The disorders of rhythm caused by an ectopic focus are solitary extrasystoles, a paroxysmal, or a continuous disorder of rhythm. The focus may be supraventricular, causing atrial and nodal tachycardia, or it may be ventricular and give rise to ventricular tachycardia. Atrial fibrillation and

flutter probably also result from a rapidly discharging supraventricular ectopic focus (Annotation, *Lancet*, 1954). There is a special risk of systemic embolism in atrial fibrillation, and of ventricular fibrillation in ventricular tachycardia, but in general the effect of the tachycardia is hæmodynamic: the urgency and the outcome depend upon the rate and the condition of the heart.

High heart rates reduce the cardiac output and increase the need of the myocardium for oxygen; the reduction of the diastolic portion of every minute may cause grave disability, particularly in mitral disease; and the loss of the appropriately-timed and powerful atrial contraction may seriously embarrass the diseased ventricle and raise its diastolic pressure to 40 or 50 mm Hg (Fig. 13.1). The coronary blood flow may be greatly reduced by the fall in aortic pressure, the rise of left ventricular diastolic pressure towards the diastolic arterial level, and the shortening of the diastolic intervals. In healthy young people tachycardia may be merely a nuisance, but in patients with heart disease it may cause pulmonary œdema from elevation of the left heart diastolic pressure, congestive heart failure, cardiac pain, hypotension and peripheral vascular constriction, oliguria or anuria, metabolic acidosis and death. The hæmodynamic effect of dysrhythmias has been described at length by McIntosh & Morriss (1966).

Julian, Valentine & Miller (1964) examined the disturbances of heart rate, rhythm and conduction that occurred in 100 consecutive unselected patients with acute myocardial infarction, of whom 31 died. They observed that "The disturbance of rate, rhythm and conduction in acute myocardial infarction are related to prognosis in a number of different ways. Firstly, there are those (such as bundle branch block) which merely reflect extensive myocardial damage and have no deleterious effects. Secondly there are some (for example atrial flutter) which, while associated with severe disease, lead to a further deterioration in cardiac function by decreasing coronary blood flow and systemic arterial pressure. Thirdly, there are those (such as complete heart block and ventricular fibrillation) that are often fatal yet may occur without great cardiac damage. Fourthly, some disorders (such as frequent ectopic beats) have serious significance only because they herald fatal arrhythmias. Fifthly, there are those disturbances that are commonly the result of treatment (such as the Wenckebach type of heart block). When affected patients die, this is not a result of the conduction disturbance but may be due to either the treatment or the serious disease that required it. Finally, there are the arrhythmias (for example supraventricular ectopic beats) which are provoked by the myocardial infarction but are of no consequence. Many of the arrhythmias affect the prognosis in more than one of these ways." These authors also pointed out the great risk of ventricular fibrillation in cases where ventricular ectopics occur during the T wave of the preceding heart cycle.

Before selecting treatment of an ectopic dysrhythmia it is necessary to consider the general factors that may be playing a part, the condition of the heart, and the severity of the effects of the dysrhythmia as well as its nature. The general factors may include exertion, pain and fear, anoxia, hypotension, metabolic acidosis, infection and intoxication, and thyrotoxicosis which can be difficult to detect in the elderly. The treatment is influenced by the nature, the severity and the stability of the intrinsic heart disease. For example, the

infarcted myocardium is more than usually sensitive to digitalis, particularly when oral diuretics have been used; quinidine should be avoided, if possible, in posterior infarction with its high risk of heart block; and beta-adrenergic blocking agents are dangerous in the presence of heart failure and atrio-ventricular conduction defects. The severity of the effects of the dysrhythmia may be assessed from the examination of the CVP, the arterial pressure, the peripheral blood flow, the urine flow, the radiographic demonstration of pulmonary oedema, and from the measurement of the arterial blood's oxygen saturation and tension, carbon dioxide tension and pH. The nature of the ectopic rhythm is usually diagnosed from a routine electrocardiogram, but an oesophageal lead or skilled examination of the jugular pulse and heart sounds may be needed.

The correction of general factors and the provision of reassurance and rest are important in the prevention and treatment of the ectopic rhythms. Sedation with promethazine hydrochloride and pethidine may be extremely helpful, and a trial of droperidol and phenoperidine has shown promising results. Morphine may cause harm by reducing the peripheral resistance and increasing venous pooling (Henney *et al.*, 1966). In many cases the barbiturate hypnotics are satisfactory but they may cause hypotension when the patient is nursed in the head-up position. Oxygen therapy is desirable. In severe cases the legs should be raised to encourage venous return, and the head lowered to provide protection against reduction of the cerebral flow.

### **The Prevention of Ectopic Rhythms and their Sequelæ**

It is not a simple matter to select drugs to prevent the occurrence or recurrence of ectopic rhythms.

The danger of unheralded ventricular fibrillation resulting from the use of quinidine sulphate has caused this drug to lose favour. However, Lown (1966) uses it prophylactically in acute cardiac infarction, and Julian, Valentine & Miller (1964) believe that it should be used when there is a high risk of ventricular fibrillation, for example in the presence of ectopic beats of the R on T type. Cramer, Varnauskas & Werko (1963) have introduced a slow-release preparation of quinidine bi-sulphate ("Kinidin Durules", Astra) and it is suggested there is little chance of the blood concentration rising to a dangerous level with a dose of 500 to 750 mgm twice daily. Julian, Valentine & Miller (1964) have pointed to the special danger of quinidine in posterior cardiac infarction where there is a high risk of heart block.

Procaine-amide (Kayden, 1965), like quinidine, has a depressant and anti-arrhythmic effect, and similarly is contra-indicated in heart block. It is commonly used for the prevention of ventricular tachycardia, and a daily dose of 3 to 6 g is usually effective.

Antazoline (Dreifus *et al.*, 1963) and lidocaine (= xylocaine) (Frieden, 1965) have been introduced, and are likely to gain a wider acceptance.

Digitalis intoxication may readily appear after cardiac infarction, heart surgery or artificial ventilation, particularly when oral diuretics have been used, and cause dangerous ectopic tachycardia or ventricular fibrillation. These may require treatment with potassium if the beta-adrenergic blocking agents are contra-indicated. In an urgent situation, 25–50 mEq potassium



(1.8 to 3.7 g potassium chloride) can be given by intravenous drip over a period of two hours. The potassium chloride should be diluted in, say 500 ml of five per cent glucose. If there is a suspicion of impaired renal function potassium should be given with the greatest caution, and only when strongly indicated. For oral use Arnott (1966) has recommended the preparation SlowK (Ciba). Potassium should not be given when heart block is a toxic manifestation of digitalis (Fish, Martz & Priebe, 1960).

✓ The beta-adrenergic blocking agent propranolol may be extremely dangerous in incipient or actual heart failure (Stock & Dale, 1963; Wolfson, Robbins & Krasnow, 1966) and cause hypotension or heart block (Sloman, Robinson & McLean, 1965). Its use is contra-indicated in diffuse obstructive airway disease (Rowlands, Howitt & Markman, 1965) and in bronchospasm, bradycardia and atrioventricular dissociation (Wolfson, Robbins & Krasnow, 1966). Propranolol appears to reduce ectopic activity (Stock & Dale, 1963; Rowlands *et al.*, 1965; Wolfson, Robbins & Krasnow, 1966) and to slow the ventricular rate in atrial fibrillation and flutter (Stock & Dale, 1963; Rowlands, Howitt & Markham, 1965; Wolfson, Robbins & Krasnow, 1966). It appears to have a particular place in the digitalis-induced dysrhythmias (Stock & Dale, 1963; Williams & Sekiya, 1963; Sloman, Robinson & McLean, 1965; Wolfson, Robbins and Krasnow, 1966); and perhaps in quinidine intoxication (Seaton, 1966). It is of value in resistant and recurrent ventricular fibrillation (Sloman, Robinson & McLean, 1965). Sloman, Robinson & McLean (1966) also believe that propranolol may be of value as a prophylactic agent in patients with mild cardiac infarction. In emergencies the minimal effective dose of propranolol should be given intravenously, and Besterman (1965) recommends starting with 1 mg.

When frequent ectopic activity is associated with sinus bradycardia it may sometimes be abolished by using atropine, or a slow intravenous infusion containing isoprenaline to produce a sinus tachycardia of 100 to 110 per minute; or by injecting xylocaine slowly intravenously. Normally the maximum dose of xylocaine does not exceed 1 mg per kg of body weight. Sodi-Pallares (1961) suggested the use of potassium combined with glucose and insulin in the treatment of acute cardiac infarction because this was thought to favour the entry of potassium into injured heart muscle cells. Mittra (1965) made a controlled trial with a modified regime and suggested that it lowered mortality by reducing the incidence of ventricular fibrillation. Nixon, Ikram & Morton (1966a and b), who have treated cardiogenic shock with an infusion of glucose alone, and found their patients were remarkably free of dysrhythmia during the infusion, suggested that the effect of the glucose on the potassium mechanism might be a partial explanation for this finding.

Artificial pacing of the heart by means of a catheter-tip electrode introduced into the right ventricle has proved successful in the suppression of rapid ventricular arrhythmias (Sowton, Leatham & Cardon, 1964). The application of double and triple electrical impulses to control the heart rate and protect the patient from the life-threatening tachycardias which are resistant to drugs and countershock has been used successfully, but the technique is fraught with danger. The subject has been described by Lopez, Edelist & Katz (1963); Braunwald and co-workers (1964); Bayley & Lightwood (1966) and Hoffman & Cranefield (1966).

### **The Correction of Ectopic Rhythms**

When the ectopic rhythm causes little disturbance and does not threaten life it may suffice to apply the general treatment that is described above, and allow the disorder to subside in its own time. In emergencies it may be desirable to use countershock without delay. In the majority of cases the urgency lies between these extremes, and treatment with drugs is indicated.

In the supraventricular tachycardia digitalis may be used to reduce the rate of the ventricles and it must be remembered that the effective dose and the margin of safety can be reduced greatly by cardiac infarction, heart surgery, the prolonged use of oral diuretics, potassium depletion and artificial ventilation. After digitalis has been given carotid sinus pressure may abolish an ectopic rhythm when previously it had been ineffective. Once the ventricular rate has been controlled with digitalis sinus rhythm may return spontaneously, or be obtained with countershock at a convenient time. Digitalis may however cause dangerous dysrhythmias and sudden death without any warning such as gastro-intestinal disturbance or bradycardia (Fig. 13.11). Atrial tachycardia is a particularly dangerous manifestation which should be suspected if the heart rate increases during treatment with digitalis or the rhythm becomes regular in a patient who previously had atrial fibrillation (Matthews, 1960).

➤ In most cases of ventricular tachycardia procaine amide is a satisfactory drug. It may be given orally, intramuscularly or intravenously. When the intravenous route is chosen, doses of 100 mg may be given at intervals of not less than one minute and the blood pressure should be measured frequently. If the blood pressure falls it should be allowed to rise again before the next dose is given.

In the ill patient an ectopic tachycardia may sometimes be abolished by gently raising the blood pressure for a short period with a small dose of a pressor amine.

Sometimes the intravenous injection of procaine amide or xylocaine will terminate a supraventricular tachycardia, particularly one of recent onset. It is probable that the effective dose of xylocaine depresses the heart and peripheral resistance less than that of procaine amide (Austen & Moran, 1966).

Parasympathetic stimulants such as acetyl-choline cause cardiac arrest, and they have no place in the treatment of ectopic tachycardia in ill patients.

There is neither need nor justification for patients to be subjected to the hazards of quinidine for the conversion of a dysrhythmia to sinus rhythm. It should be emphasized that patients may die suddenly and unexpectedly from this drug even when the precautions of admitting the patient to hospital, giving a test dose, digitalizing beforehand, eliminating heart-failure, and continuously monitoring the ECG have been taken (Davies, Leak & Oram, 1965).

### **CARDIOVERSION**

Cardioversion is a new electrical method of terminating diverse ectopic abnormalities of the heart beat, and it has been widely adopted since Lown, Amarasingham & Neuman (1962) introduced their technique of applying a modified capacitor discharge across the chest wall during the safe period of

the cardiac cycle. Their technique for depolarizing the heart avoids the usual hazards of electricity, namely, asystolic arrest and ventricular fibrillation. For a detailed consideration of the various points that must be considered in clinical practice the reader is referred to Lown (1964), Oram & Davies (1964), Korsgren and co-workers (1965), and Paulk & Hurst (1965).

Cardioversion is used in the emergency treatment of the life-threatening ectopic dysrhythmias, and electively in less urgent clinical situations. It is safer than conversion with quinidine, and does away with the anxious period of close observation and ECG monitoring that were required when quinidine was used. Quinidine's depression of myocardial contractility and reduction of the peripheral resistance precludes its use in the patients who are ill with heart failure and hypotension, but cardioversion may be applied to these cases who require urgent treatment.

### Indications

In atrial flutter cardioversion appears to be the treatment of choice. It should also be used when ventricular tachycardia causes pulmonary oedema and hypotension in cardiac infarction. In cases where the ventricular tachycardia is well tolerated, procaine amide may be tried first. The drug should be abandoned for cardioversion if 1 g given intravenously is ineffective, or causes hypotension or QRS widening.

Cardioversion is indicated in the supraventricular tachycardias which are resistant to carotid sinus pressure, digitalis and procaine amide. It is strongly indicated where the absence of the atrial pumping action disables the diseased ventricle and causes refractory heart failure or severe angina pectoris.

Oram & Davies (1964) give the following list of indications for the cardioversion of atrial fibrillation:

1. Atrial fibrillation threatening life:
  - (i) Refractory failure from rapid ventricular rate uncontrolled by digitalis.
  - (ii) History of embolism.
2. Atrial fibrillation persisting after cause removed or alleviated:
  - (i) Surgical:
    - (a) Mitral valvotomy.
    - (b) Thyroidectomy for thyrotoxicosis.
    - (c) Pericardectomy for constrictive pericarditis.
    - (d) Repair of atrial septal defect.
  - (ii) Medical:
    - (a) Failed drug therapy in thyrotoxicosis.
    - (b) Alcoholic cardiomyopathy.
    - (c) Pneumonia, virus myocarditis.
    - (d) Cardiac infarction.
3. Atrial fibrillation without demonstrable heart disease ("*lone*" fibrillation).
4. Mitral valve disease too slight to warrant surgery.
5. Rheumatic heart disease not amenable to surgery.

### Contra-indications

The contra-indications to cardioversion include severe and uncorrected mitral valve disease with atrial fibrillation present for more than a year, tight mitral stenosis awaiting valvotomy, giant enlargement of the left atrium, calcification of the left atrial wall and complete heart block. Digitalis therapy is discussed below.

### The Preparation for Cardioversion

Before elective cardioversion is carried out it is desirable to remove as many of the contributory causes of the dysrhythmia as possible, and to wait until the pattern of dysrhythmia has become stable. Electrolyte disturbances, particularly potassium depletion, must be corrected.

With regard to digitalized patients, it should be emphasized that the degree of digitalization which is sufficient to control the ventricular rate without evidence of toxicity before the cardioversion may be sufficient to cause death from dysrhythmia after the cardioversion. Moreover, cardioversion is not effective in the digitalis-induced dysrhythmias (Lown, Kleiger & Williams, 1965; Gilbert & Cuddy, 1965). It follows that the dosage of digitalis should be reduced or stopped some days before the cardioversion. Quinidine is given before cardioversion by Lown (1964) and Paulk & Hurst (1965), but not by Oram & Davies (1964) or Korsgren and co-workers (1965). Opinions differ with regard to the value of anticoagulant treatment before conversion: Paulk & Hurst (1965) believe that it makes little difference to the risk of embolism; Lown (1964) gives it for three weeks to patients with a history of embolism and to young women with asymptomatic mitral disease and atrial fibrillation of recent onset; Oram & Davies (1964) give it to cases of mitral stenosis, patients with large hearts, and where the atrial fibrillation is of recent onset. Korsgren and co-workers (1965) give anticoagulants to every case.

Most workers prefer light anaesthesia with a drug having a brief action, e.g. methohexitone sodium, 50 to 120 mg intravenously. Premedication is generally avoided because it may lead to unrecognized hypoventilation at a time when maximal oxygenation is desirable (Paulk & Hurst, 1965).

### The Conversion

The ECG electrodes should be placed on the limbs as far from the chest as possible to prevent the possibility of arcing, and the lead should be selected which gives the maximal QRS voltage and minimal base-line variation. The electrodes used for the application of the shock should be applied to the front and the back of the chest, with the heart in between them. The shock should not be given before the base-line of the ECG monitor has become stable, nor *before test shocks have been fired to ensure that the discharge is delivered within the safe period of the cardiac cycle, that is to say, during the down-slope of the R wave or the nadir of the S wave.* The initial energy setting for the discharge is 50 watt-seconds. If this is ineffective, successive discharges can be given without delay. The next discharge is at 100 watt-seconds and thereafter the energy is increased by increments of 100 watt-seconds to a maximum of 400.

After the conversion, Oram & Davies (1964) give digitalis to make sure that the ventricular rate is slow if atrial fibrillation recurs. They do not use quinidine after conversion because of the risk of sudden death, but Korsgren and co-workers (1965) believe the drug is needed to prevent a high rate of relapse. Lown (1964) uses it. It is to be hoped that the slow-release preparation of quinidine bisulphate (500–750 mg b.d.) may provide an effective preparation sufficiently free from risk for routine use.

It should be remembered that the gaining and the retention of sinus rhythm require not only an electrical discharge that is adequate to depolarize the heart, but also an internal, physiological capacity of the sinus node to unction. The health of this mechanism cannot be diagnosed before conversion, and its loss can only be inferred from the failure of the adequately prepared and selected patient to maintain sinus rhythm. It should be noted also that the atrium may not contract detectably when the P wave first returns to the electrocardiogram (Logan *et al.*, 1965). Atrial mechanical activity may not even develop for several days, and in some cases it seems never to return. Anticoagulant therapy should probably be continued until the mechanical contraction of the left atrium can be detected with a suitable pulse transducer applied to the chest wall over the apex of the left ventricle (Ikram, Nixon & Arcan, 1966).

As complications of cardioversion, pulmonary oedema occurs with extreme rarity (Resnekow & McDonald, 1965), and a variety of dysrhythmias may appear for a short time after the shock. Complications tend not to occur when digoxin is stopped 48 hours before cardioversion and electrolyte disorders are corrected, when the lowest effective energy is used for the shock, when a suitable E.C.G. lead is used for monitoring, when test shocks are applied to test the synchronizing mechanism, and when premedication and deep anaesthesia are avoided.

### HEART-BLOCK

Heart-block is most commonly encountered as a complication of acute cardiac infarction. If the patient lives the conduction defect almost always disappears within a fortnight. Less commonly, transient heart-block occurs in non-specific myocarditis, various fevers and in acute rheumatism. It may result from digitalis and quinidine poisoning, and from hyperkalæmia. Permanent heart-block is usually caused by sclerosis of the left side of the cardiac "skeleton" and rarely by coronary disease, tumours or syphilis (Lev, 1964).

Heart-block may be associated with intractable heart failure, inadequate cerebral blood flow or angina pectoris which can be relieved only by the provision of an adequate ventricular rate, but the commonest indications for treatment are attacks of loss of consciousness and the risk of sudden death.

In intermittent heart-block the attacks of loss of consciousness may occur during the transition from sinus rhythm. In permanent heart-block they may result from extreme bradycardia, from atrial standstill with ventricular asystole or from paroxysmal ventricular tachyarrhythmia. Before deciding upon treatment it is desirable in every case to monitor the E.C.G. in an attempt to determine the cause of the syncope because, while the sympathetico-mimetic drugs may be used in some cases (Linenthal & Zoll, 1963), they may

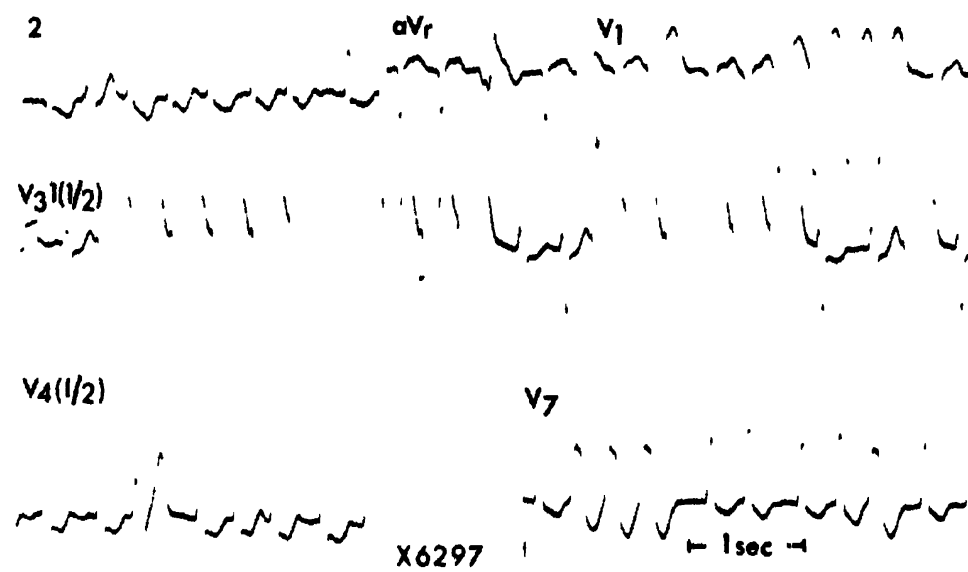


FIG. 13.11. ECG leads recorded before treatment with potassium in a patient with digitalis intoxication precipitated by diarrhoea after oral diuretics had been taken daily for a long period.

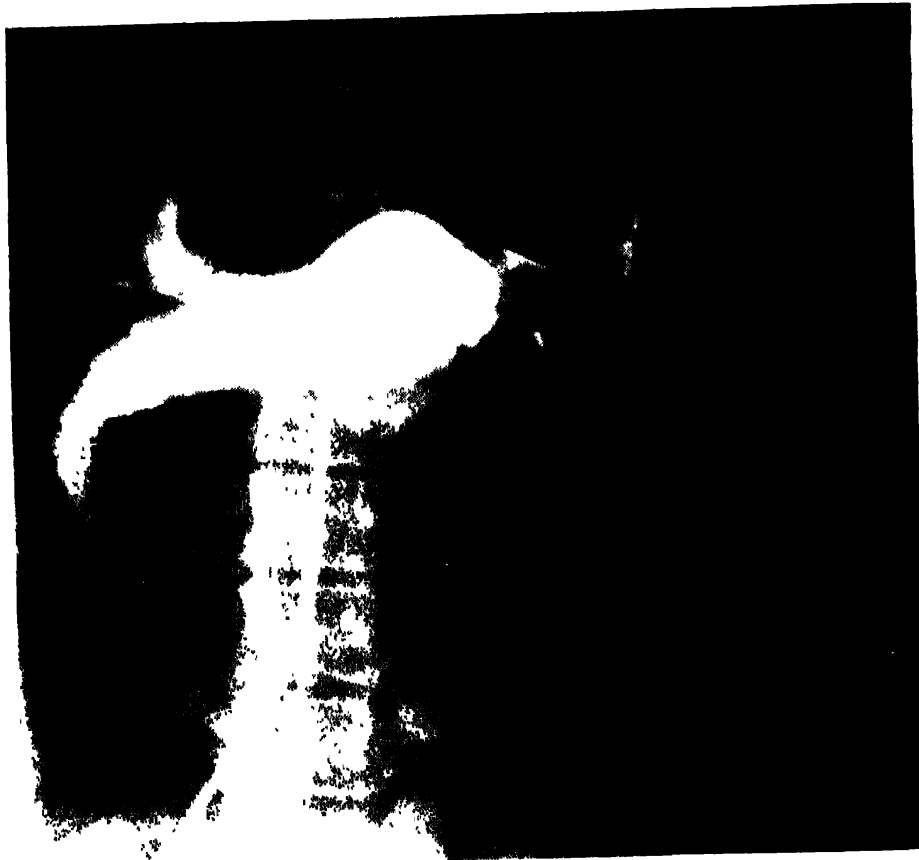


FIG. 13.13. The pulmonary angiogram from a patient with almost total obstruction of the left main pulmonary artery. A little contrast medium reaches the upper lobe.

endanger patients with paroxysmal ventricular tachyarrhythmia. Procaine amide and quinidine should never be used in the latter cases because of the high risk of their causing prolonged asystole (Bluestone & Harris, 1965).

In the non-urgent case isoprenaline tablets (10 mg) may be taken under the tongue as often as required. For no clear reason the heart-block is better controlled in some patients by rectal suppositories containing 10 or 15 mg isoprenaline. About 30 per cent of patients are amenable to treatment with a long-acting preparation of isoprenaline ("Saventrine") (Fleming & Mirams, 1963; Bluestone & Harris, 1965).

In emergencies an intravenous drip is put up and the slow ventricular rate corrected with an infusion of five per cent glucose in water containing 3 mg of isoprenaline per 100 ml, or 3 ml 1/1,000 adrenaline per 500 ml (Dimond, Dunn & Brosius, 1960). Landgren & Bjork (1963) prefer isoprenaline for cases of permanent complete heart-block and adrenaline for the unstable varieties. The infusion is given at the slowest effective rate. A constant watch must be kept on the ECG monitor to guard against the hazard of ventricular tachyarrhythmia. Inadvertently increasing the drip-rate may cause ventricular fibrillation. An electrical pacemaker should be used as soon as possible. A platinum-tipped catheter is introduced through the median cubital vein into the right ventricle under fluoroscopic control and a ventricular rate of 80 to 90 per minute is obtained with an electrical pacemaker. It is important to avoid the risk of mains-voltage-induced ventricular fibrillation by adequately earthing the pacemaker and any electrocardiograph that may be attached to the patient. A portable battery-driven pacemaker allows the patient to leave his bed.

If the heart-block requires permanent pacing the catheter is left in position for a week; during this time the stability of the tip's position in the ventricle can be ensured and the optimal heart rate determined. Then the patient is taken to the operating theatre and an adequate heart rate is maintained with an intravenous drip of isoprenaline in glucose solution. The catheter is amputated in the antecubital fossa. The axilla is opened. The cut end of the catheter is drawn into the wound through an incision into the axillary vein and connected to a pacemaker unit which is implanted behind the pectoral fold. Gold, Paneth & Gibson (1966) invented this elegant technique for endocardial pacing.

Many workers prefer exocardial pacing in which wires are attached to the surface of the heart at thoracotomy.

The implantable pacemaking equipment now available is adequate for the purposes of preventing sudden death and maintaining active life but a great deal of improvement is needed in the design of the battery units and the construction of the wires and catheters.

### CARDIAC TAMPONADE

A collection of fluid in the pericardium tends to prevent the ventricles from filling. Large amounts accumulating over a long period may be tolerated well but even a small amount, say of 100–200 ml, may be dangerous if the accumulation is rapid. Collection of fluid may result from the dissection of an aortic aneurysm, from rupture of a cardiac infarct or aneurysm, from penetrating wounds of the heart and from pericarditis. In urgent cases the



patient may be thought to collapse without warning but usually warning is given by the signs of distress, rising jugular venous pressure, falling arterial pressure and a paradoxical pulse (Fig. 13.12). Examination of the heart sounds and pulsation may not be helpful in making the diagnosis. It may be confirmed radiologically when the pericardial outline is seen to be at some distance from the right atrial border, which may be defined by placing a cardiac catheter against it, or by injecting contrast medium or carbon dioxide into the right atrium.

Most workers who are accustomed to inserting needles in and around the heart prefer to make a diagnostic aspiration through the fifth left intercostal space in the mid-clavicular line (Milstein, 1963; Hollman, 1966) with a 20 or 21 swg needle. The needle is less rigidly held by the tissues than it is when the epigastric route is followed, there is no risk of tearing the impalpable wall of

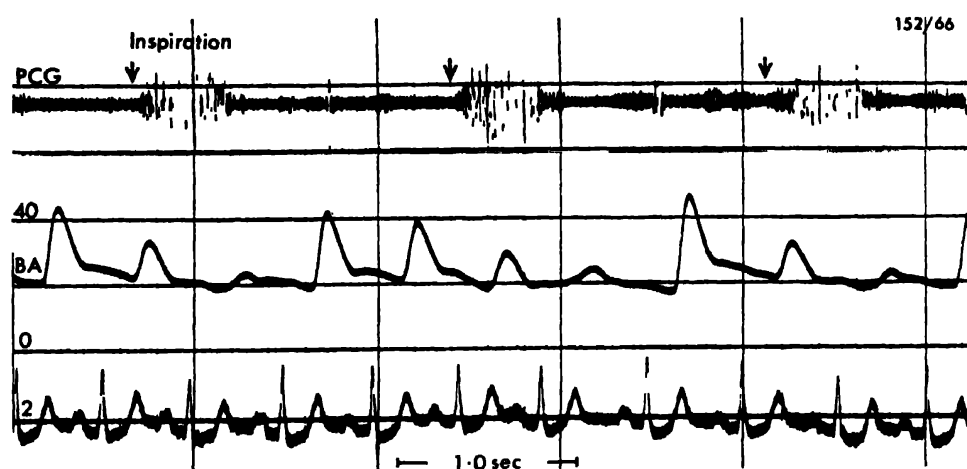


FIG. 13.12. The paradoxical arterial pulse in a patient with tamponade. The upper tracing shows the noise of inspiration as recorded by a microphone on the chest wall.

the right ventricle, the heart apex can be felt to touch the point of the needle if approached, and branches of the coronary artery are not likely to be severed by the needle tip.

The treatment of acute tamponade is almost always surgical, the pericardial sac being emptied at thoracotomy.

### PULMONARY EMBOLISM

The introduction of portable heart-lung machines primed with dextrose solution instead of fresh blood is providing a new approach to the problem of acute pulmonary embolism and recent experience suggests that many of our concepts need refashioning. (The suggestions tentatively put forward here stem from experience shared with Mr. A. R. Makey.)

The patient with hæmoptysis, pleuritic pain and a wedge-shaped shadow on the lung X-ray probably has severe disease of the left side of the heart; he probably has little capacity to survive pulmonary embolectomy, and operation carries the hazard of severe bleeding from the infarcted area.

The patient who may be saved by pulmonary embolectomy has usually been in good health before developing phlebothrombosis after an acute illness or operation, and may complain of difficulty in breathing, oppression

in the chest or palpitation. There is a sudden collapse, or hour-by-hour deterioration. The jugular venous pressure rises and the arterial pressure falls; there may be pallor and sweating. There is a palpable parasternal heave over the right ventricle and the heart has a triple rhythm from the addition of a pathological fourth heart sound.

The electrocardiogram may show acute clockwise rotation, or one of the other patterns of right heart "strain". If a chest radiograph of good quality can be obtained it may show a reduction of the vascular markings and increased translucency in the affected area. Catheterization of the right heart may reveal only slight or moderate pulmonary hypertension with gross elevation of the right atrial and right ventricular end-diastolic pressures.

In urgent cases, where time permits, a pulmonary angiogram is carried out while the preparations for operation are being made. The contrast material is injected into the vena cava, the right ventricle or the pulmonary artery. This investigation has the advantage of confirming and localizing the embolism with less risk than thoracotomy, and its practice produces surprising results. For example, it is learned that patients with a confident clinical diagnosis of massive pulmonary embolism may have some other condition such as acute myocarditis or circulatory overloading from excessive infusion. Beall & Cooley (1965) have also encountered acute cardiac infarction. The small embolism with which the patient presents may be seen to be the last of a series that has silently obstructed most of the pulmonary arterial tree (Fig. 13.13, p. 403).

Makey considers it desirable to cannulate the inferior vena cava from the leg, and the femoral artery, in order that the circulation may be supported by a heart-lung machine at an early stage of the operation. He believes that a median sternotomy provides the best approach. Three of his cases of acute pulmonary embolectomy were completely successful. His other two cases did not survive operation and these were patients with heart failure from long standing ischæmic heart disease.

It is important that these diagnostic and surgical techniques should be developed because massive pulmonary embolism often kills the young and healthy (Fleming & Bailey, 1966).

### **CORONARY-CARE UNITS; INTENSIVE-OBSERVATION AND INTENSIVE-CARE UNITS**

The care of acute cardiac cases requires first a group of nurses who are highly trained in a general way and especially trained in techniques that vary from the close observation of the patient who is at the moment free from complications, and in need of peace and quiet, to the intensive-care of the seriously ill patient whose life is repeatedly threatened by a rapidly-moving train of complications. A second need is a place that is adequately provided with equipment and organized for fast and efficient work. Such a unit may be equally useful for a wide range of severe illnesses, particularly for acute respiratory failure, coma and recovery from open-heart surgery. A suitable unit should provide a suite of rooms, adjacent to the operating theatres and the cardiac department. The requirements of the ward may be considered under four headings, namely the patients, the nursing functions, the ward design and the equipment.

### The Patients

Two conditions are foremost: firstly that the patient was capable of working before the onset of the severe and acute illness, injury, intoxication or operation, and secondly, there is a high probability of return to work if the care is successful.

### Nursing Functions

These fall into four categories: general nursing, monitoring, technical and the provision of skilled assistance.

The *general nursing* has to be of a high standard and patience and kindness are particularly desirable.

The *monitoring functions* are important because the electrocardiogram is the only physiological variable which lends itself to consistent and reliable instrumental monitoring at the present time. Consequently the other variables have to be monitored by the nurses. They include:

- (a) Distress—from anxiety
  - from pain
  - from a fall in blood volume
  - from early left heart failure
  - from airway obstruction
  - from bladder distension.
- (b) Cyanosis.
- (c) The peripheral circulation e.g. coldness and blueness with high venous pressure indicates heart-failure or tamponade, and guttering of the peripheral veins indicates reduction of the blood volume.
- (d) The balance of the blood and fluid intake and output.
- (e) The electrocardiogram. The nurse should be able to recognize the common disorders that appear on the oscilloscope and know how and when to take a written record. She should be able to judge the severity of a dysrhythmia from its effect on the peripheral circulation, the venous pressure and the arterial pressure.
- (f) The venous pressure: the use and care of the saline manometer.
- (g) The arterial pressure. The nurse should understand the indications for frequent readings and the signs that suggest the patient does not need to be disturbed by the use of the sphygmomanometer. She should understand the use and the care of the electromanometer and recorder.

The *technical functions* required of the nurses themselves include:

- (a) An understanding of the changes in the central venous pressure in relation to over and under-transfusion and heart-failure; and in the arterial pressure in relation to the effects of sedative drugs and posture.
- (b) The treatment of cerebral oedema, with an understanding of the need for cooling, dehydration and an optimal posture.
- (c) Artificial respiration and closed-chest cardiac massage and the use of the electrical defibrillator.
- (d) The operation of pacemaking equipment.

- (e) The use of venous and arterial pressure recording equipment and E.C.G.-monitoring oscilloscopes and electrocardiographs.
- (f) Artificial ventilation: the use of ventilators; care of indwelling endotracheal tubes and tracheostomy; humidification; tracheo-bronchial toilet.

The nurse should be able to give *skilled assistance* to medical staff in the performance of venous, arterial and cardiac catheterization and pacemaking; tracheostomy; peritoneal and renal dialysis; and know how to handle isotopes safely.

### The Ward Design

The various rooms in the suite contain from one to four beds according to the needs of the cases and the requirements for apparatus. The rooms should look like wards rather than laboratories with provision for adequate storage and shelf-space, floor-space, illumination, power-outlets, oxygen and suction. Racks and sliding hooks eliminate the need for drip stands. An "intercom" provides for normal and emergency communication. Trunking in the wall permits the inexpensive modification of electrical systems.

Adjacent to the ward are situated the operating theatres and the cardiac department which provides special services and equipment from its blood-gas, respiratory function, biochemical and heart-catheterization laboratories and its electronics workshop. The other needs of the ward are provided by a ventilator servicing and sterilization area, the hospital laboratories and services and the Central Sterile Supply Department. Sleeping and washing accommodation is required for the medical staff and accommodation for relatives.

### The Equipment

In addition to the normal medical and surgical ward stock and the equipment provided from the Cardiac Department's laboratories, the acute ward requires equipment for the different varieties of intravascular catheterization, for resuscitation, cardioversion, pacemaking, artificial ventilation, bronchoscopy and tracheostomy, ECG-monitoring and for hypothermia.

In the United States it is expected that provision of coronary-care units will save 45,000 lives each year. The considerations involved in the creation of these units are described in the report of the Second Bethesda Conference of the American College of Cardiology (1965).

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## APPENDIX 1

### FOR THE INFORMATION OF ALL REGISTRARS, HOUSE OFFICERS AND WARDS INSTRUCTIONS FOR THE TREATMENT OF CARDIAC ARREST

A proportion of deaths from cardiac arrest can be prevented by prompt treatment because the heart may stop from temporary electrical instability or conduction defect without there being irreversible myocardial damage.

These conditions are: Ventricular fibrillation; asystolic arrest; heart block.

The treatment must be given promptly if cerebral damage is to be avoided during the period of circulatory arrest.

#### The Treatment

- Phase I* If sufficient helpers are available and the patient is not connected to drips or drainage suction he/she should be placed on the floor. Otherwise the board provided in every ward should be placed on the bed and the patient rolled on to it.  
N.B. The floor is the better position.  
Lower the head and clear the airway.  
Raise the legs.  
Thump the middle of the sternum once.

In a number of cases this treatment will restore the heart beat.

- Phase II* Begin closed chest cardiac massage and also begin mouth to mouth artificial respiration (an Ambu bag or Brooke airway should be used if available).

Done properly this will keep the patient in a safe condition for an hour.

Once instituted, send someone else to get the Telephonist to put out the Resuscitation call for the medical assistance which is now essential.

- Phase III* Intubate the trachea and continue ventilation  
Cut down on a vein (preferably in the ankle) and erect a drip of 5 per cent dextrose in water.  
Record an ECG

- Phase IV* *Definite Treatment (Cardiac Arrest Trolley)*  
Give 50 ml 8.4 per cent sodium bicarbonate and 10 ml 10 per cent calcium gluconate (into the drip tubing for dilution).  
Give 1 ml 1/1,000 Adrenaline HCl (into the drip tubing for dilution) if small magnitude ventricular fibrillation is present.  
Defibrillate externally or apply the pacemaker as necessary.

IT IS IMPORTANT TO CONTINUE THE MASSAGE AFTER THE HEART FIRST BEGINS TO BEAT UNTIL THE HEART'S OWN BEAT IS STRONG ENOUGH TO PRODUCE A GOOD PULSE AND TO KEEP THE PUPILS SMALL.

- Phase V* *Supportive Treatment*  
A respirator may be necessary.  
Isoprenaline or adrenaline may be necessary.

**IF CONSCIOUSNESS DOES NOT QUICKLY RETURN:**

Use a respirator.

Elevate the head of the bed if the blood pressure is adequate.

Give intravenous urea after catheterizing the bladder.

Cool the patient to 32–33°C (rectal) by giving promazine hydrochloride (Sparine), covering with a wet sheet, and blowing with fans.

**IF FITS OCCUR:**

Control them initially with the minimal effective dose of intravenous thio-pentone.



## APPENDIX 2

### CONTENTS OF THE WARD BOXES

Baxter Giving Set.  
Portex Red i/v Catheter.  
Steriflex Sodium Chloride Soln. 0·9 per cent one litre.  
Dextrose five per cent 500 ml in Glass bottle.  
Ureaphil. 80 g.  
Brooke Airway.

*Drugs:*      Injections of:  
                 Adrenaline 1" 1,000 1 ml  $\times$  4.  
                 Isoprenaline 5 mg/10 ml  $\times$  2.  
                 Noradrenaline Acid Tartrate 8 mg/4 ml. (Noradrenaline  
   base 4 mg)  $\times$  1.

*Suppositories.* Isoprenaline 10 mg  $\times$  3.

Cut Down Set—Consisting of:  
                 Bard-Parker Handle No. 4.  
                 Aneurysm Needle.  
                 Spencer-Wells Forceps—Straight.  
                 Spencer-Wells Forceps—Curved.  
                 One pair Fine Pointed Scissors.  
                 Dissecting Forceps.  
                 Fine Toothed Forceps.  
                 Hamilton Bailey Cannula.  
                 One curved Butting Needle.

Portex, Male Gibbon Catheter Size 12.  
5  $\times$  No. 13 Gauze Squares—Sterile.  
5  $\times$  No. 1 Wool Balls—Sterile.  
50 ml. 8·4 per cent Sodium Bicarbonate Solution.  
One Roll 1 in. Oxide Strapping.  
5  $\times$  No. 1 Needles.  
5  $\times$  No. 12 Needles.  
5  $\times$  No. 20 Needles.

*Drugs:*      Injections of:  
                 Aminophylline 0·25 g/10 ml  $\times$  1.  
                 Atropine 0·6 mg/1 ml  $\times$  1.  
                 Calcium gluconate 10 per cent 10 ml  $\times$  2.  
                 Methoxamine HCl. (Vasoxine). 20 mg/1 ml  $\times$  1.  
                 Procaine Amide 100 mg/10 ml  $\times$  1.  
                 Thiopentone 0·5 g  $\times$  1  
                 Water for Injection 10 ml  $\times$  1.  
                 Water for Injection 20 ml  $\times$  1

Ampoule File  $\times$  1.  
1  $\times$  20 ml Syringe.  
1  $\times$  10 ml Syringe.  
3  $\times$  5 ml Syringe.  
5  $\times$  2 ml Syringe.

## **APPENDIX 3**

### **CONTENTS OF EMERGENCY INTUBATION BOX**

**Cardiff Bellows, Pulmonator, or Resusci Folding Bag, with face masks.**

**1 Laryngoscope with 1 spare blade.**

**3 Cuffed endotracheal tubes, each with nosworthy connector.**

**Cuff inflation syringe and clip.**

**Airways.**

**Female nosworthy connector and corrugated tube.**

**1 tube lubricating jelly.**

**1 packet gauze swabs.**

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